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NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

FY 1992 ANNUAL REPORT

OCTOBER 1, 1991 THROUGH SEPTEMBER 30, 1992

NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

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DIVISION OF INTRAMURAL RESEARCH

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 22109-04 CMB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Comparison of Tissue Response to Complete Freund's Adjuvant and Newer Adjuvants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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Others: W. S. Stokes Chief, Comparative Medicine Branch CMB, NIEHS

P. H. Myers Biol Lab Tech, VM CMB, NIEHS

J. A. Clark Biol Lab Tech, VM CMB, NIEHS

COOPERATING UNITS (if any)

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LAB/BRANCH

Comparative Medicine Branch

SECTION

Veterinary Medicine

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

.3

PROFESSIONAL:

.1

OTHER:

.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study is to determine an adjuvant that will maximize antibody response and minimize animal pain and distress, thereby benefitting the experimental animal and improving the scientific result. Tissue reactions and antibody titers were compared using two common adjuvants, complete Freund's adjuvant and Ribi adjuvant, in rabbits. Results indicate that both adjuvants produce tissue reactions at the site of inoculation. Reactions caused by RIBI adjuvant were similar in nature but reduced in severity. It appears that RIBI adjuvant produces acceptable antibody titers when the antigen is a large protein (e.g. 700 kDa), but significantly lower levels than complete Freund's adjuvant when a small protein is used as antigen. These findings are consistent with recent reports (Deeb et al., Comparative antibody response and health effects using Ribi and Freund's adjuvants in rabbits, AALAS Abstract, 1991; Johnston et al., An evaluation of several adjuvant emulsion regimens for the production of polyclonal antisera in rabbits, AALAS Abstract, 1991).

The next adjuvant that will be evaluated is Hunters TiterMax. Hunters TiterMax, a water in oil emulsion, is the newest adjuvant that has been marketed as an alternative to complete Freund's adjuvant. TiterMax contains no protein, bacterial or plant components, or Mycobacterium that would cause hypersensitivity reactions or granulomas. Rabbits will be clinically evaluated for pain and distress, and gross and histopathologic collections will be made. Antibody titers will also be evaluated and compared with complete Freund's adjuvant. These findings may help substantially in realizing the goal of an acceptable alternative to complete Freund's adjuvant.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES-22110-04 CMB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Alopecia and Dermatitis in C57BL/6N Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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M. F. Goelz Veterinary Medical Officer, VM CMB, NIEHS

J. Locklear Vet Med Ofcr/Microbiologist, QAL CMB, NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Comparative Medicine Branch

SECTION

Quality Assurance Laboratory

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

.3

PROFESSIONAL:

.1

OTHER:

.2

CHECK APPROPRIATE BOXES

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

C57BL/6N mice, an important strain used in NIEHS research, develop an alopecia which usually arises at 4 to 6 months of age. This condition often progresses into a protracted dermatitis and may become severe enough that the animals develop ulcerative skin lesions and suffer premature morbidity and mortality. This study was directed at discovering a nutritional basis for the progressive skin disorders.

Mice were divided into groups of 15 and fed either the standard NIH-31 control diet or NIH-31 test diet fortified at three times the normal concentration with either animal tallow, menhaden oil, safflower oil, soybean oil, thiamine, riboflavin, pyridoxine, cyanocobalamin, biotin choline, vitamin E, zinc oxide or other vitamin or mineral mixtures. Mice were evaluated at 3-month intervals for quality of hair coat, alopecia, dermatitis, and skin lesions. Mice were sacrificed when initial skin lesion developed. Preliminary analysis of these results suggest that the alopecia and degenerative skin conditions were reduced in groups of mice fed the test diet fortified with the B vitamins (B₁, B₂, B₆), Vitamin E, Biotin, or Choline when compared to the groups of mice fed the control diet. After one year, the mice fed the B vitamins, Vitamin E, Choline and Biotin had healthier, thicker hair coats and more mice survived as compared to the control groups. These preliminary findings suggest that C57BL/6N mice may require dietary changes as they age to compensate for natural degenerative skin changes that occur, probably due to strain dependent genetic predisposition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES-22111-01 CMB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Research Complication of Streptococcus agalactiae Infections in Nude Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: M. F. Goelz Veterinary Medical Officer CMB, NIEHS

Others: W. S. Stokes Chief, Comparative Medicine Branch CMB, NIEHS

P. H. Myers Biol Lab Tech, VM CMB, NIEHS

J. A. Clark Biol Lab Tech, VM CMB, NIEHS

J. E. Thigpen Microbiologist, QAL CMB, NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Comparative Medicine Branch

SECTION

Veterinary Medicine Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

.3

PROFESSIONAL:

.1

OTHER:

.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Group B Streptococcus agalactiae is a pathogen for nude mice which have been stressed by experimental manipulation and/or fighting. Recent reports have indicated this agent is also a pathogen in immunocompetent mice and rats. The purpose of this study was to determine whether the type of drinking water consumed by nude mice exacerbated or inhibited the incidence of streptococcal septicemia or abscess formation in nude mice. One hundred nude mice were cultured on arrival for Group B streptococci. Mice were divided into 4 groups based on the type of drinking water offered. The water included tap, hyperchlorinated, acidified and neomycin-treated acidified water. Mice were observed daily for signs of septicemia and necropsied immediately. Blood was drawn for complete blood count and bacterial culture. Spleen, liver, kidney, and heart were cultured and taken for histopathological examination. Cases of streptococcal septicemia were present in all groups. Preliminary results indicate that the type of drinking water does not influence the incidence of streptococcal septicemia or abscess formation in nude mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES-22112-01 CMB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Anesthetic and Pathologic Effects of Tribromoethanol in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	M. F. Goelz	Veterinary Medical Officer, VM	CMB, NIEHS
Others:	W. S. Stokes	Chief, Comparative Medicine Branch	CMB, NIEHS
	P. H. Myers	Biol Lab Tech, VM	CMB, NIEHS
	J. A. Clark	Biol Lab Tech, VM	CMB, NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Comparative Medicine Branch

SECTION

Veterinary Medicine

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

.3

PROFESSIONAL:

.1

OTHER:

.2

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Tribromoethanol (TBE) is an injectable anesthetic agent widely used in mice and rats. Many reports indicate that this is a good anesthetic with no side effects. Other reports have stated that TBE supplies adequate anesthesia, but side effects (i.e., peritonitis, intestinal ileus) are sequelae to administration. Decomposition of TBE or a second administration of any anesthetic including TBE have been incriminated as causing these lesions. The purpose of this study was to determine the gross and microscopic changes that occur at various time periods following intraperitoneal administration of TBE to CD-1 mice at a dose commonly cited in the literature. In addition, the anesthetic properties will be evaluated. Four groups of mice received either TBE, TBE and amylene hydrate, amylene hydrate or saline by intraperitoneal injection. The depth of anesthesia was evaluated. Mice were euthanized from 1 day post-administration to 89 days post administration. At necropsy, blood was taken for complete blood count. Peritoneal lavage was done for cytological and microbiological evaluation. Tissues were taken for histopathology. Results of our study to date indicate that TBE can produce an anesthetic state in mice, but it is inconsistent and often variable. Post-administration complications have included mild transient peritonitis. This controlled study documented that this anesthetic produces consistent mild peritoneal inflammation that could potentially adversely affect the scientific objectives of studies in which this agent is used. Additional testing may be necessary to determine if there is an appropriate dose that produces reliable anesthesia and analgesia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80001-20 LCMP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Microsomal Mixed-Function Oxidase System: Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Richard Philpot	Sup. Research Chemist	LCMP	NIEHS
Kave Nikbakht	Sr. Staff Fellow	LCMP	NIEHS
Michael Lawton	IRTA Fellow	LCMP	NIEHS
Emmanuel Atta-Asafo-Adjei	Visiting Fellow	LCMP	NIEHS

COOPERATING UNITS (If any)

University of California, Davis, CA; Scripps Institute and Research Foundation, La Jolla, CA; North Carolina State University, Raleigh, NC.

LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

SECTION

Molecular Pharmacology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

7.0

PROFESSIONAL:

4.0

OTHER:

3.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

During the past several years this laboratory has concentrated on studies of the drug-metabolizing enzyme, the flavin-containing monooxygenase (FMO). Thought to comprise two forms at the most, we have discovered that the FMO is a gene family with at least five. Recently, we have begun to express these enzymes in COS-1, yeast, and *E. coli* systems in order to examine their substrate specificities and other properties. The properties of two of the enzymes, formerly called the "liver" and "lung" forms, in expression systems are the same as those observed with purified preparations with the exception of their kinetic behavior which is much less complex for the expressed enzymes. A third form that has been expressed exhibits unique substrate specificity in that it appears to metabolize only primary amines. However, neither hydrogen peroxide nor hydroxylamine equivalents are formed by the reaction. It is suspected that the hydroxylamine formed is further metabolized to the oxime. One notable common property among the sequences of the five FMOs is the inclusion of two putative pyrophosphate binding domains, one near the N-terminus and the second near residue 190. These areas, which account for the binding of FAD and NADPH to the enzyme are proposed to be critical for catalytic activity. We have modified the first of these sites by changing the GxGxxG sequence into GxGxxV and expressed the altered protein. This protein is devoid of activity and does not appear to have any FAD bound to it. A second notable common property among the five forms of FMO is the presence of an extremely hydrophobic C-terminal peptide comprising the final 26 amino acids. It has been proposed that the enzyme is anchored to the membrane via this peptide. We have examined the properties of the FMO expression product missing the C-terminal 26 residues. Results of this work demonstrate conclusively that the C-terminal hydrophobic peptide does not anchor the enzyme to the membrane. Future work will concentrate on the development of antibody preparations for each form of the FMO and on the elucidation of the physiological roles of these enzymes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80031-16 LCMP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Altered Membrane Function in Xenobiotic Toxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

John B. Pritchard	Research Physiologist	LCMP	NIEHS
David S. Miller	Research Physiologist	LCMP	NIEHS
Natascha A. Wolff	Visiting Fellow	LCMP	NIEHS
Donna E. Stewart	Staff Fellow	LCMP	NIEHS

COOPERATING UNITS (if any)

University of Florida; Duke University

LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

SECTION

Comparative Membrane Pharmacology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

6.0

PROFESSIONAL:

3.0

OTHER:

3.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transport of solutes across polar epithelia is required for function of many organs, e.g., kidney. In turn, epithelial transport depends upon the coordinated function of transport proteins in apical and basal poles of these cells. In recent years, we have examined the mechanism and energetics of renal organic anion (OA) transport, the major system which governs the elimination of foreign chemicals, demonstrating its indirect coupling to metabolic energy through Na/ α -ketoglutarate (α KG) co-transport and OA/ α KG exchange. Our current focus is on the intracellular and luminal events associated with OA transport, the mechanism(s) of OA transport in extrarenal tissues, and the molecular biology of the OA system. Isolated luminal membrane vesicles show both anion exchange and carrier-mediated, potential-driven pathways. However, only the potential-driven pathway appears to mediate luminal exit of secreted OA. Interestingly, during secretion OA show punctate accumulation within the cytoplasm. This process is energy dependent and saturable. Its relation to net transport is under investigation. Extrarenal tissues (liver and choroid plexus) also appear to utilize indirect Na coupling to drive OA transport. However, this appears to be substrate specific, e.g., fluorescein, but not 2,4-dichlorophenoxyacetic acid, in the choroid plexus and taurocholate, but not bile pigments, in the liver, show Na/ α OG coupled transport. The basis for these differences is under study. Finally, initial steps in expression cloning of the renal OA transport proteins have been completed and a cDNA library is currently being screened to identify clones which carry nucleotides encoding these transport proteins. Once isolated these nucleotides will provide probes for investigation of the control, development, and distribution of this important excretory transport system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80042-06 LCMP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Calcium Regulation and Signal Transduction Mechanisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

James W. Putney, Jr.	Chief	LCMP	NIEHS
Frank Menniti	Senior Staff Fellow	LCMP	NIEHS
Gary Bird	Visiting Fellow	LCMP	NIEHS
Kerry Oliver	Senior Staff Fellow	LCMP	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

SECTION

Calcium Regulation Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

5.58

PROFESSIONAL:

3.58

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The broad aim of this project is to understand at the cellular and molecular level the mechanisms by which surface membrane receptors for hormones, neurotransmitters and growth factors modify cellular responses through mobilization of cellular Ca^{2+} . An early event in the action of receptors of this class is the hydrolysis of a membrane lipid, phosphatidylinositol 4,5-bisphosphate to a Ca^{2+} -mobilizing second messenger, which releases Ca^{2+} from an intracellular organelle. The general approach in this project is to combine HPLC measurements of the formation and metabolism of inositol phosphates with real time measurements of cytosolic Ca^{2+} using intracellular fluorescent Ca^{2+} indicators. Our previous work has shown that a mechanism exists for signalling the entry of Ca^{2+} into cells following the depletion of Ca^{2+} from an intracellular organelle through the action of IP_3 . We are now currently attempting to understand the molecular mechanism underlying this process utilizing as a model that Ca^{2+} -mobilizing actions of the tumor promoter, thapsigargin, which activates this process while bypassing surface receptors and the generation of IP_3 . We have also recently discovered a novel technique for imaging intracellular Ca^{2+} pools in living cells, and are currently investigating changes in intracellular Ca^{2+} storage compartments during cellular signalling. Since the Ca^{2+} signalling system is centrally involved in the regulation of cellular growth under normal and pathological (neoplastic) conditions, these studies may provide novel perspectives on the pharmacological regulation and arrest of these processes. In addition, Ca^{2+} is believed to play a central role in mechanisms of chemically-induced cell injury, and thus these studies should provide insights into the mechanisms underlying the pathophysiological consequences of exposure to toxins and other environmental agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80043-05 LCMP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ion Channel Modulation by Signal Transduction Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David Armstrong	Sr. Staff Fellow	LCMP	NIEHS
Anatoly Shcherbatko	Visiting Associate	LCMP	NIEHS
Richard White	Sr. Staff Fellow	LCMP	NIEHS

COOPERATING UNITS (if any)

Dr. A. Schonbrunn, Dept. Pharmacology, UT School of Medicine, Houston, TX; LMIN, NIEHS.

LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

SECTION

Calcium Regulation Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

3.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have used patch clamp techniques to investigate the effects of two important classes of inhibitory neuropeptides on electrical excitability of the molecular level in a tumor cell line from rat pituitary. Both classes of neuropeptides inhibit dihydropyridine-sensitive calcium channels and stimulate charybdotoxin-sensitive potassium channels by reversing the effects of cyclic AMP-dependent phosphorylation at a step distal to cyclic AMP synthesis or degradation. Experiments with phosphatase inhibitors and purified enzymes implicate the polycation-stimulated phosphoserine protein phosphatase 2A as the final common effector on both types of channel proteins. Natriuretic peptides activate phosphatase 2A through a cyclic GMP-dependent protein kinase. Many other neuropeptides, like somatostatin, that act through pertussis toxin-sensitive G-proteins, stimulate phosphatase 2A through a lipoxigenase metabolite of arachidonic acid. We are currently pursuing the generality of these novel and important inhibitory signalling pathways.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80045-04 LCMP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

GTP-Binding Proteins and Signal Transduction: Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Martin Rodbell	Sr. Research Scientist	LCMP	NIEHS
Katsuya Nagai	Visiting Fellow	LCMP	NIEHS
Saleem Jahangeer	Visiting Scientist	LCMP	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

SECTION

Signal Transduction Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

3.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous studies established that GTP-binding regulatory proteins (G-proteins) can be extracted from hepatic and brain membranes with octylglucoside as large structures (>12S) suggestive of multimers. Exposure of these membranes to activating guanine nucleotide (GTPγS) alone or with hormone (glucagon, with liver) caused the large structures to break down into structures similar in size to heterotrimeric G-proteins. Several types of G-proteins were shown to have different size multimers depending on the type of detergent used for extraction; each G-protein type displayed distinct sensitivity to the "depolymerizing" actions of GTPγS. Synaptoneurosomes treated with p-phenylenedimaleimide were shown to contain cross-linked G-proteins that, on sizing columns, resemble the polymeric forms of actin and tubulin in these membranes. These findings coupled with the detergent studies and target size analysis of adenylyl cyclase systems suggest that membrane receptors are linked to multimeric structures of G-proteins. A new concept of hormone action is proposed which takes into account the multimeric structures of G-proteins and which suggests that the coupling and dynamics of receptor/G-protein interactions are controlled by the binding and hydrolysis of GTP, analogous to the ATPase-controlled interactions of multimeric actin and myosin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

FORM 10-1 (10-1988)

Z01 ES 80046-04 LCMP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Inositol Lipid Signalling Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Stephen B. Shears	Visiting Scientist	LCMP	NIEHS
Munir Abdullah	Sr. Staff Fellow	LCMP	NIEHS
Andrew Craxton	Visiting Fellow	LCMP	NIEHS
Nawab Ali	Staff Fellow	LCMP	NIEHS

COOPERATING UNITS (If any)

Dr. Glenn Prestwick, State University of New York; Dr. Traynor-Kaplan, UCSD Medical Center, California; Dr. Keith Nye, University of London, United Kingdom.

LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

SECTION

Inositol Lipid Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

3.6

PROFESSIONAL:

3.6

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Activation of cell-surface receptors releases inside cells Ins(1,4,5)P₃, which mobilizes cellular Ca²⁺ stores as part of a ubiquitous signalling cascade. This project studies the enzymes metabolizing Ins(1,4,5)P₃, since this deactivates signalling, while introducing down-stream metabolites such as Ins(1,3,4,5)P₄, that may themselves have key functions. We have purified and characterized an active Ins(1,3,4,5)P₄ 3-phosphatase and shown both this activity, and Ins(1,4,5)P₃ 5-phosphatase, to be perturbed in some cases of cell transformation, and following HIV infection of lymphocytes. We are pursuing the mechanisms of these effects. With the aim of understanding the physiological significance and mechanisms behind receptor-regulated InsP₃ turnover (itself a novel effect recently uncovered in this laboratory), two major enzymes of InsP₃ synthesis have also been highly purified: Ins(3,4,5,6)P₄ 1-kinase and Ins(1,3,4)P₃ 6-kinase, both with the help of a novel InsP₃-affinity column. With an underlying goal of studying the impact of environmental toxins on signalling processes, we have made progress in elucidating the mechanisms by which aluminum toxicity may manifest itself; although our work shows no direct effect upon Ca²⁺ signalling, we have discovered this ion may redirect the synthesis of the cell's complement of InsP₃ and InsP₄. We have also discovered that low concentrations of fluoride can regulate the interconversion of inositol polyphosphates with a novel metabolic group of inositol pyrophosphates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80047-03 LCMP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Intracellular Receptors and Metabolic Control

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David S. Miller	Research Physiologist	LCMP	NIEHS
David M. Barnes	Staff Fellow	LCMP	NIEHS
James J. Smith	Staff Fellow	LCMP	NIEHS

COOPERATING UNITS (if any)

Michigan Cancer Foundation; Weizmann Institute

LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

SECTION

Intracellular Regulation Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

3.5

PROFESSIONAL:

2.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Complex organisms use chemical signals, e.g., hormones and growth factors, to integrate and coordinate the function of specialized tissues. Information is transferred to target cells when chemical signals bind to specific surface receptors and, as a result, cell structure and function can be profoundly altered. This project is concerned with events that occur after such signals are received, intracellular events that transmit the information from the surface receptor to the cell's biochemical machinery. To study how information flows to metabolic control points, we have developed a powerful experimental system, consisting of a giant cell (amphibian oocyte), paraffin oil-based cell microinjection and microdissection procedures and single cell analyses. Recent experiments have focussed on the actions of insulin and certain heavy metal salts (vanadate and Zn⁺⁺), which, at low concentrations, act as insulin mimics. We found that a peroxide of vanadate (pervanadate) rather than vanadate itself is the form of the ion that stimulates hexose uptake and protein synthesis. In addition hexose uptake, but not protein synthesis, is stimulated by microinjected, intracellular pervanadate. Pervanadate stimulates protein synthesis (but not hexose uptake) by a Mg-dependent mechanism, increasing Mg influx at the surface membrane. Since translation is particularly sensitive to intracellular Mg, increased influx appears to signal increased translation. Future plans include: 1) determining the detailed mechanisms by which external and internal pervanadate affect the function of oocytes and somatic cells, 2) examining the role of intracellular Mg in metabolic control, and 3) determining how other heavy metal ions, which act as insulin mimics at low concentrations, disrupt cellular control mechanisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80048-02 LCMP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular Mechanisms of Xenobiotic Transport

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David S. Miller	Research Physiologist	LCMP	NIEHS
John B. Pritchard	Research Physiologist	LCMP	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

SECTION

Intracellular Regulation Section and Comparative Membrane Pharmacology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An important function of the vertebrate kidney is the excretion of potentially toxic chemicals, such as waste products of cellular metabolism, xenobiotics and xenobiotic metabolites. This occurs primarily in the proximal tubule, where renal transport systems remove organic anions and organic cations from peritubular capillaries and transport them across the tubular epithelium into the lumen. Much work has focussed on the mechanisms by which organic anions and organic cations are transported across the surface membranes of the proximal tubular cells, but little attention was paid to the routes by which these compounds cross the cell interior, the assumption being that simple diffusion across the cytoplasm was the only process necessary. We are using comparative renal models (proximal tubules from lower vertebrates and invertebrates and renal cells in culture) in combination with fluorescence microscopy (conventional and confocal), video imaging and intracellular microinjection to test that assumption. We have found that, after transport into renal cells, organic anions are distributed over two compartments: one that is diffuse and cytoplasmic, and a second that is punctate and vesicular. Sequestration in intracellular vesicles is not a result of endocytosis, but rather of uptake from the cytoplasm. Such uptake is concentrative, specific and energy-dependent. These findings indicate that the intracellular distribution of organic anions is not uniform and that, since a substantial fraction of cellular organic anions are sequestered, the average cellular concentrations measured in most experiments greatly overestimate cytoplasmic concentrations. Future studies will focus on: 1) characterizing the mechanism of vesicular organic anion sequestration in renal cells, 2) determining if organic cations are also sequestered and by what mechanism, 3) defining the role sequestration plays in overall renal xenobiotic secretion, and 4) searching for analogous intracellular secretion mechanisms in other specialized epithelia that accumulate and transport organic anions and cations, e.g., choroid plexus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 61024-10 LG
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (Title must fit on one line between the borders) Genetic and Molecular Analysis of <i>suppressor of sable</i> Function in <i>Drosophila</i>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: R. A. Voelker Others: W. R. Steinhauer J. F. Sterling J. P. Graves W. Gibson S. R. Lamprides/T. V. Garland/A. Barnes S. K. Jarchow/M. G. Sheridan	Research Geneticist IRTA Biologist Biologist Chemist Biological Aid (SIS) Summer IRTA	LG, NIEHS LG, NIEHS LG, NIEHS LG, NIEHS LG, NIEHS LG, NIEHS LG, NIEHS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Genetics		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS: 4.3	PROFESSIONAL: 1.2	OTHER 3.1
CHECK APPROPRIATE BOXES!		
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK		
We are investigating the molecular mechanism of action of the <i>suppressor of sable</i> [<i>su(s)</i>] system of <i>Drosophila melanogaster</i>. Recessive <i>su(s)</i> mutations suppress recessive second-site mutations that are caused by insertions of the mobile elements 412 and P in 5' transcribed but nontranslated sequences. Current evidence suggests that this suppression is effected by some mechanism that increases the amount of pseudo-wild-type mRNA that is produced by splicing the mobile element sequences from the primary transcript. The <i>su(s)</i> cDNA contains an open reading frame that encodes a putative polypeptide of 1322 amino acids, and cellular fractionation studies have shown that the protein is primarily located in the nucleus. This protein has only one region of general similarity to other proteins: a highly charged region that is similar to a putative nuclear localization signal found in several human, <i>Xenopus</i> and <i>Drosophila</i> proteins, all of which are known to be RNA binding proteins. Males and females lacking the entire <i>su(s)</i> protein are viable and fertile, albeit markedly below the levels of viability and fertility of wild-type flies. Because the <i>su(s)</i> protein has very limited similarity to other proteins, the important functional domains of the protein are being identified by determining which regions of the protein have been evolutionarily conserved. The <i>su(s)</i> gene of the distantly related <i>Drosophila virilis</i> has been cloned and partially sequenced, and it contains both regions of high homology and of considerable divergence. We are also attempting to clone the <i>su(s)</i> gene from the very distantly related flour beetle, <i>Tribolium castaneum</i>, for similar studies.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61043-02 LG

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)

Mammalian Lactate Dehydrogenase Genes and Their Encoded Isozymes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Steven S.-L. Li Research Geneticist LG, NIEHS

Others: M. A. Qureshi Fulbright Scholar LG, NIEHS

COOPERATING UNITS (if any)

Dr. M. Maekawa, Hamamatsu University School of Medicine, Japan

LAB/BRANCH

Laboratory of Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.3

PROFESSIONAL:

0.3

OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☒ (b) Human tissues

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK

The molecular nature of many genetic mutations resulting in deficiencies or electrophoretic variants of human LDH-A or LDH-B isozyme has been illustrated. The LDH-A deficiency is a latent heredity disorder of exertional myopathy. Female patients frequently complain of uterine stiffness during pregnancy, and delivery by Caesarean section is necessary.

This information is of value to improve health care, particularly of women, and it will also allow more accurate evaluation of genetic mutation events caused by environmental mutagens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61044-02 LG

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)

Research Services on Protein Sequencing and Peptide Synthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Steven S.-L. Li

Research Geneticist

LG, NIEHS

COOPERATING UNITS (if any)

Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina

LAB/BRANCH

Laboratory of Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.2

OTHER

CHECK APPROPRIATE BOXES

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK

The Protein Chemistry Laboratory of NIEHS and UNC-CH has provided research services on protein microsequencing and peptide synthesis to a number of scientists at the NIEHS as well as UNC-CH. Several new genes have been cloned and antigenic determinants of some proteins characterized as the result of these investigations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 61045-02 LG
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT <i>(Title must fit on one line between the borders)</i> Human Prostatic Acid Phosphatase Gene and Its Encoded Enzyme		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i>		
PI: Steven S.-L. Li	Research Geneticist	LG, NIEHS
Others: Farida S. Sharief	Biologist	LG, NIEHS
COOPERATING UNITS <i>(if any)</i> Dr. T. M. Chu, Roswell Park Memorial Institute, Buffalo, New York		
LAB/BRANCH Laboratory of Genetics		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS: 1.3	PROFESSIONAL: 0.3	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☒ (b) Human tissues		
SUMMARY OF WORK Human prostatic acid phosphatase (PAP) has been used for diagnosis of prostate cancer, the most commonly found cancer in United States males. Human genomic clones containing PAP gene have been isolated and the exon-intron organization of PAP gene has been illustrated. The expression of human PAP gene, as well as PSA (prostatic specific antigen) gene, in the benign and cancerous prostate tissues has been investigated by Northern blot analysis.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 61046-02 LG
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT <i>(Title must fit on one line between the borders)</i> Mammalian Protein Tyrosine Phosphatase Genes and Their Encoded Isoenzymes		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i>		
PI: Steven S.-L. Li	Research Geneticist	LG, NIEHS
Others: Hitoshi Miyasaka Esther Hou	Visiting Fellow Biologist	LG, NIEHS LG, NIEHS
COOPERATING UNITS <i>(if any)</i>		
LAB/BRANCH Laboratory of Genetics		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS: 2.3	PROFESSIONAL: 1.3	OTHER 1.0
CHECK APPROPRIATE BOX(ES)		
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither		
☐ (a1) Minors		
☐ (a2) Interviews		
SUMMARY OF WORK		
Protein tyrosine phosphatase (PTP) may play a key role in controlling cell growth and proliferation as well as possible in tumor suppression. Two different cDNAs encoding PTP have been isolated from mouse testis and T-cell cDNA libraries, and their nucleotide sequence determined. The expression of these PTP-1 and PTP-2 genes from different mouse tissues was investigated by Northern blot analysis. The PTP-1 gene was found to be abundant in testis as well as brain.		

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 ES 61047-02 LG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)

Mutagenesis in *in vivo* Models of Deficient Xenobiotic Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. G. Burkhardt	Chemist	LG, NIEHS
	H. V. Malling	Research Geneticist	LG, NIEHS
Others:	K. S. Sampson	Biologist	LG, NIEHS
	K. K. Ferris	Guest Researcher	LG, NIEHS

COOPERATING UNITS (if any)

E. J. Eisen, NCSU, Raleigh, North Carolina
D. Casciano, NCTR, Jefferson, Arkansas

LAB/BRANCH

Laboratory of Genetics

SECTION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

INSTITUTE AND LOCATION

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.5

OTHER

0.5

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

The basis for this project is the hypothesis that certain individuals with normally benign moderate deficiencies in metabolism of xenobiotic compounds may be predisposed to high risk from exposure to carcinogens, mutagens, or hormone therapy. The hypothesis will be tested using a new and unique mouse mutation that we have identified and characterized as a deficiency in UDP-glucuronosyltransferase activity; the enzyme is part of a multisubstrate family responsible for metabolism of many endogenous and exogenous compounds. The mutation is identified by an autosomal recessive transient neonatal jaundice. The deficiency is nonacute and lifelong; Gilbert's syndrome is a close parallel in the human population. We have determined that recessive females have elevated estradiol levels and that affected individuals of both sexes have liver hyperplasia associated with increased mitosis, cytomegaly, and single-cell necrosis; a pleiotropic characteristic that has particular interest is the presence of many hepatic eosinophilic (preneoplastic) foci. Our goals are to 1) determine the effect of the deficiency on spontaneous and induced mutagenesis by producing transgenic animals containing a marker for mutation and 2) ask whether deficient individuals are hypersensitive to tumorigenesis. If there is marked hypersensitivity, then these animals may be a useful genetic model for defining factors involved in progression. Mice carrying the recessive enzyme deficiency will be made transgenic for a mutation marker and used to investigate the potential for increased sensitivity to both mutagenic and carcinogenic agents among homozygous-deficient individuals in comparison to their litter mates with normal levels of activity. Two different markers for mutation have been prepared for evaluation of specific mutational endpoints. The mouse line has been caesarean-rederived and made pathogen-free in preparation for the transgenic insertion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61048-02 LG

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)
Genetic and Molecular Analysis of *Minute(1)B* Function in *Drosophila*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	R. A. Voelker	Research Geneticist	LG, NIEHS
Others:	W. R. Steinhauer	IRTA	LG, NIEHS
	C. N. Rogers	Summer IRTA	LG, NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH Laboratory of Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 0.8	OTHER 0.7
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CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

In *Drosophila melanogaster* there exists a class of mutations called "Minutes," which is characterized by phenotypic abnormalities caused by defective protein synthesis. While several of these loci have been identified as encoding ribosomal proteins, others are likely to encode other components of the protein synthesizing apparatus. The *Minute(1)B* [*M(1)B*] gene is likely a member of the latter category, in that its putative polypeptide product has a molecular weight of ~125 KD, much larger than the largest ribosomal protein (~50 KD). From cloning and sequencing the *M(1)B* gene, we have identified an open reading frame that could encode a 1133 amino acid polypeptide. This protein has no homology to any known protein sequences or to proteins that could be produced by the GenEMBL nucleic acid data base. Cellular fractionation and immunological techniques utilizing polyclonal antisera made against three nonoverlapping regions of the open reading frame have localized the protein in a subregion of the nucleus, possibly the nucleolus. This suggests that the protein may have some function in ribosome biogenesis. The protein is produced at high levels during oogenesis, at low levels during early embryogenesis and at increasing levels in later stages of embryogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 61049-02 LG	
PERIOD COVERED October 1, 1991 to September 30, 1992			
TITLE OF PROJECT (Title must fit on one line between the borders) Enzymology of Homologous Recombination and DNA Repair in <i>Drosophila melanogaster</i>			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)			
PI:	Miriam Sander	Senior Staff Fellow	LG, NIEHS
Others:	Mark Nugent	Summer IRTA	LG, NIEHS
	Shu-Mei Huang	Geneticist	LG, NIEHS
	Liya Gu	Visiting Fellow	LG, NIEHS
	Meryl Carter	Biologist	LG, NIEHS
COOPERATING UNITS (if any)			
LAB/BRANCH Laboratory of Genetics			
SECTION			
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709			
TOTAL STAFF YEARS:	3.65	PROFESSIONAL:	1.70
		OTHER	1.95
CHECK APPROPRIATE BOXES)			
☐ (a) Human subjects		☐ (b) Human tissues	
☐ (a1) Minors		☒ (c) Neither	
☐ (a2) Interviews			
SUMMARY OF WORK			
We have purified and characterized a <i>Drosophila</i> protein called Rrp1 (Recombination Repair Protein 1), which is a homologue of <i>Escherichia coli</i> exonuclease III based on the similarity of their amino acid sequences. Rrp1 is enzymatically similar to its bacterial homologue, both when purified from <i>Drosophila</i> embryos and when expressed in <i>E. coli</i> from its cDNA. The protein copurifies with apurinic endonuclease and dsDNA 3'-exonuclease. The apurinic endonuclease activity has been demonstrated using partially depurinated plasmid DNA as a substrate. The Rrp1 AP endonuclease specific activity is 1.2×10^5 U/mg, a value that is less than two-fold different than the exonuclease III specific activity. The exonuclease activity of Rrp1 requires a base-paired terminal nucleotide and is 3'-5' specific. Surprisingly, the ratio of these two activities is dramatically different for Rrp1 protein and its bacterial homologue. In addition to having DNA repair functions, Rrp1 carries out <i>in vitro</i> the DNA strand transfer step that is important in homologous recombination and catalyzes the renaturation of ssDNA. A single peptide with an apparent size of 105 kDa copurifies with each of the enzymatic activities of Rrp1. This polypeptide is sufficient for all these activities, since a highly purified protein fraction synthesized in <i>E. coli</i> demonstrates electrophoretic, chromatographic and enzymatic properties similar to those of native <i>Drosophila</i> Rrp1 protein. The Rrp1 protein family includes <i>E. coli</i> exonuclease III, <i>S. pneumoniae</i> exonuclease A, bovine BAP, mouse APEX endonuclease, and human APE. Within a 250 amino acid region colinear homology is shared between all members. Rrp1 is unique in that it includes an N-terminal region of more than 400 amino acids for which no related sequence has been found. This region is thought to interact with ssDNA and is likely to influence the enzymatic properties of Rrp1. Rrp1 may facilitate recombinational repair at sites of DNA damage, or may have distinct and independent functions in homologous recombination and DNA repair.			

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 61050-02 LG
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT <i>(Title must fit on one line between the borders)</i> Investigation of Germinal Mutation and Morphogenesis in Mice		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i>		
PI: F. M. Johnson	Research Geneticist	LG, NIEHS
Others: M. L. Snell	Biologist	LG, NIEHS
COOPERATING UNITS <i>(if any)</i> Dr. D. P. Lovell, British Industrial Biological Research Association, Carshalton, Surrey, England		
LAB/BRANCH Laboratory of Genetics		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5
CHECK APPROPRIATE BOXES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK Our interests in this project involve the effects of mutation on development. We have used the mouse skeleton as a model system. Unusual animals are identified by making a number of measurements on a variety of bones. More severe effects are indicated by greater numbers of animals with more extreme measurements. Collectively, measurements define size and shape. At the level of DNA and chromosome structure, the physical nature of induced mutations varies considerably depending on the dose, the reactive properties of the chemical and other factors. Our approach endeavors to provide a quantitative measure of biological damage which can be used to evaluate the genetic impact of germinal mutations induced by different environmental agents.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 61051-01 LG
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT <i>(Title must fit on one line between the borders)</i> The Genetics of Adaptation to Contaminated Environments		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)</i>		
PI: F. M. Johnson	Research Geneticist	LG, NIEHS
Others: M. L. Snell	Biologist	LG, NIEHS
COOPERATING UNITS <i>(if any)</i>		
LAB/BRANCH Laboratory of Genetics		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 0.5	OTHER 0.5
CHECK APPROPRIATE BOXES:		
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither		
☐ (a1) Minors		
☐ (a2) Interviews		
SUMMARY OF WORK		
Organisms vary in their sensitivity to toxic environmental chemicals, depending on genetic and other factors. Genetic variability implies that the structure of exposed populations of plants and animals will change from one generation to the next, based in part upon degree of sensitivity. Sensitive genotypes will be replaced by resistant types over a period of time. Adaptation to contaminated environments could possibly have associated disadvantages. For instance, the ability to survive with a high body burden of contaminants early in life might be accompanied by organ system dysfunctions later on. We are working on methods to isolate mutations relevant to this question using the yeast <i>Saccharomyces cerevisiae</i>. We are currently looking for mutants characterized by diminished or increased ability to survive a heavy-metal-contaminated environment. We have developed a model with lead acetate that has so far produced one unusually sensitive and several resistant lines. We plan to test the model with other substances and characterize the mutations.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65033-09 LG

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)
In Vivo Mammalian Mutagenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	H. V. Malling	Research Geneticist	LG, NIEHS
	J. G. Burkhardt	Chemist	LG, NIEHS
	R. N. Winn	Biologist	LG, NIEHS
Others:	K. S. Sampson	Biologist	LG, NIEHS
	D. R. Crosslin	SIS	LG, NIEHS
	K. K. Ferris	Guest Researcher	LG, NIEHS

COOPERATING UNITS (if any)

C. A. Hutchinson, III, UNC, Chapel Hill, North Carolina
 R. J. Van Beneden, Duke University Medical Center, Durham, North Carolina
 Clement Markert, NCSU, Raleigh, North Carolina

LAB/BRANCH

Laboratory of Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.5

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK

The objective of this research is to study mutagenesis at the DNA level in mammals and teleost fish. The fish will be used for research on ova and on differentiation. A major problem in mutagenesis is that the level and specificity of response are very different between indicator organisms; predictions about induced mutagenesis may not be relevant. Significant variation is due to the diversity of marker genes; a single sequence needs to be used among various organisms and tissues. The use of integrated transgenic vector can combine a theoretical study of mutations in several model organisms with assessment of mutagenic hazard and development of biomonitoring systems for aquatic biospheres. In the initial experiments, the well-characterized *am3* of ϕ X174 has been used to evaluate substitutions at the A:T base pair. Inbred C57B1/6 mice have been made homozygous for 100 copies of ϕ X174 in a tandem array; we have not observed any detrimental effect from the insertion. Reversions via one transition and two transversions are detected by selection among progeny phage recovered from the transgenic mice. Both the recovery and the putative mutation frequencies are independent of any endogenous variation in CpG methylation among host animals or tissues. Spontaneous mutation frequencies are in the range of $2-4 \times 10^{-7}$ for liver, spleen, brain, kidney and testis. In adult animals treated with 200 mg/kg N-methyl-N-nitrosourea (ENU), there are significant increases in mutation frequencies in the spleen and liver, but not the kidney or brain. The accomplishments for the fish studies are: 1) Produced several transgenic founders of Fundulus and Medaka containing ϕ X174 as a single gene marker and 2) Demonstrated that the transgenic vector can be efficiently recovered from the chromosomal DNA of fish. We are in the process of establishing the spontaneous mutation frequency for one line of Fundulus. Efforts are underway to produce additional transgenic mice containing other specific markers for analysis of CpG-dependent and CpG-independent mutagenesis at the single gene copy level. The use of well-defined phage may allow us to compare spontaneous and induced intragenic events, recombination or developmental mosaicism in both somatic and gametogenic tissue with an overlay of comparison between distant related vertebrate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 65036-08 LG	
PERIOD COVERED October 1, 1991 to September 30, 1992			
TITLE OF PROJECT (Title must fit on one line between the borders) Gene Organization and Regulation in <i>D. melanogaster</i>			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)			
PI:	B. H. Judd	Chief	LG, NIEHS
Others:	Patricia S. Davis	Chemist	LG, NIEHS
	E. A. Goode-Montgomery	Geneticist	LG, NIEHS
COOPERATING UNITS (if any)			
LAB/BRANCH Laboratory of Genetics			
SECTION			
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709			
TOTAL STAFF YEARS:	1.50	PROFESSIONAL:	0.50
		OTHER	1.00
CHECK APPROPRIATE BOXES			
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither			
☐ (a1) Minors			
☐ (a2) Interviews			
SUMMARY OF WORK			
The project is focused on studies of gene expression during development in <i>Drosophila melanogaster</i>. Mutations of the <i>white</i> locus, which encodes a product that has strong sequence similarity with ATP-binding membrane transport proteins including the product of the cystic fibrosis gene of humans, show a wide range of changes in expression and regulation. We have concentrated on a group that originated by insertion of the transposon BEL into the 5' region of the gene. The original mutant strain and its derivatives show unusual regulatory changes in <i>white</i> expression; all have partial repression of gene expression when coupled with <i>zeste</i> mutations, a locus encoding a transcription factor. The <i>zeste</i> mutations also exhibit a trans effect on normal <i>white</i> locus function by repressing expression of paired wild-type alleles. Our results suggest that the transcription factor encoded by <i>zeste</i> functions in establishing and stabilizing the expression of <i>white</i> locus by binding DNA and modifying chromatin conformation. Another developmentally regulated locus being studied is <i>echinus</i>, a gene that plays a role in development of facets in the adult eye. The gene has been cloned and the molecular characterization is being done. A cDNA library from eye imaginal disc tissue has been probed to recover clones of the coding sequence for the protein product.			

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 65037-08 LG	
PERIOD COVERED October 1, 1991 to September 30, 1992			
TITLE OF PROJECT <i>(Title must fit on one line between the borders)</i> Transposon-Mediated Chromosome Instabilities in Drosophila			
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)</i>			
PI:	B. H. Judd	Chief	LG, NIEHS
Others:	Patricia S. Davis	Chemist	LG, NIEHS
	E. A. Goode-Montgomery	Geneticist	LG, NIEHS
COOPERATING UNITS <i>(if any)</i> Dr. John K. Lim, Distinguished Professor of Biology, University of Wisconsin, Eau Claire; and Dr. Charles H. Langley, University of California-Davis			
LAB/BRANCH Laboratory of Genetics			
SECTION			
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709			
TOTAL STAFF YEARS: 1.50		PROFESSIONAL: 0.50	OTHER: 1.00
CHECK APPROPRIATE BOXES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither			
SUMMARY OF WORK Transposons have been shown to play major roles in spontaneous mutation in Drosophila and other eukaryotes. In chromosomes, the insertion or excision of these mobile elements disrupts gene function at the site of insertion or may create deletions if excision is not precise. Additionally, recombination between two copies of a transposon situated at different sites in a chromosome or in homologs produces a variety of chromosomal rearrangements, depending on the orientation of the paired mobile elements. We are studying rearrangements produced by these types of ectopic recombination to determine the molecular structures at their breakpoint junctions and to learn more about how transposons mediate these types of asymmetrical exchanges. We have found that interchromosomal and intrachromosomal ectopic recombination are equally frequent. Homozygosity for transposon insertions and other sequence variations greatly diminishes ectopic recombination.			

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 10004-13 LMB
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>NMR Studies of the Mechanisms of Cell Injury</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute)		
PI:	Robert E. London Research Physicist	LMB NIEHS
OTHER:	Elizabeth Murphy Research Physiologist Louis A. Levy Chemist Teresa Fralix Staff Fellow	LMB NIEHS LMB NIEHS LMB NIEHS
COOPERATING UNITS (if any) Professor Charles Steenberg, Department of Pathology, Duke University, Durham, NC; Prof. Leonard S. Gettes, Dept. of Medicine, UNC Medical School, Chapel Hill, NC		
LAB/BRANCH Laboratory of Molecular Biophysics		
SECTION Nuclear Magnetic Resonance		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS: 2.8	PROFESSIONAL: 2.0	OTHER: 0.8
CHECK APPROPRIATE BOX(ES) ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The goal of this research is to elucidate the critical cellular changes that lead to irreversible cell injury. These mechanisms must be understood before rational interventions can be proposed. Previously, we have shown that an increase in cytosolic free calcium is associated with cell injury. In many systems, it has been observed that exposure of cells or animals to a brief stress provides considerable protection against a subsequent more severe stress. This brief period of stress is known to result in the synthesis of stress proteins; however, in spite of the identification of these proteins, there is little understanding of the mechanisms of protection. Exposing perfused rat heart to several brief (5 minute) periods of anoxic stress (referred to as preconditioning) affords protection when these preconditioned hearts are subjected to a longer 30-60 minute period of anoxic stress such that the preconditioned hearts have less necrosis and better recovery of function compared to untreated controls. Using ³¹P nuclear magnetic resonance (NMR) we have determined that preconditioning decreased the rate of ATP utilization; preconditioned hearts have a slower rate of decline in ATP and a reduced rate of anaerobic glycolysis. In addition, using a combination of ³¹P NMR and ¹⁹F NMR (to measure intracellular calcium) we have determined that the reduced metabolism results in less H⁺ production, and therefore less Na/H and Na/Ca exchange and ultimately less of an increase in cytosolic free calcium. We conclude that at least part of the protective effect of preconditioning is due to reduced calcium overload. We were interested in addressing the mechanism(s) responsible for the reduced rate of ATP utilization. We have investigated the hypothesis that adenosine is the mediator of preconditioning; however, in perfused rat heart adenosine does not appear to play such a role since we were unable to block the effect with an adenosine antagonist. In addition, we were not able to block preconditioning by the addition of glibenclamide, thus ruling out a role for the ATP sensitive K channels as part of this process.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30003-21 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Biochemical Methodology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Phillip W. Albro

Research Chemist

LMB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Metabolism

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.3

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

By comparing the products formed metabolically in preparations derived from washed earthworm intestinal homogenates, to those formed in preparations of individual isolates of intestinal bacteria, we are able to study the relative roles of the two species in cooperative metabolism of foreign chemicals. Selective media and differential resistance to various antibiotics are used to rapidly isolate individual bacterial species from the worms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50046-14 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Chemically Induced Photosensitivity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	Colin F. Chignell	Chief, LMB	LMB	NIEHS
	Piotr Bilski	Visiting Associate	LMB	NIEHS
	Krzysztof Reszka	Visiting Scientist	LMB	NIEHS
OTHER:	Robert H. Sik	Biologist	LMB	NIEHS

COOPERATING UNITS (if any)

Dr. William J. Lown, Dept. of Chemistry, University of Alberta, Canada; Dr. John A. Hartley, Department of Oncology, University College, London, UK

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Photobiology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.1

PROFESSIONAL:

1.6

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Light is known to interact with endogenous or exogenous chemical agents in the skin or eyes, to produce photosensitization. This phenomenon may be deleterious (phototoxicity or photoallergy) or may be useful therapeutically (photodynamic treatment of psoriasis or tumors). The objective of this project is to determine the role played by light-induced free radical species in photosensitization. In addition, efforts are directed towards the identification of photosensitizers that may be more efficient therapeutically. UV-irradiation of the photosensitizing anthraquinone-derived dye benzanthrone (7H-benz[de]-anthracene-7-one) resulted in the generation of both $^1O_2$ and O_2^{*-} in high yield. Active forms of oxygen were also implicated in the photo-killing of gram-positive bacteria by curcumin. 1,5-Diamino-4,8-dimethoxy-anthraquinone and related analogs were efficient generators of both O_2^{*-} and $^1O_2$ upon visible light illumination. These compounds were active against K562 human chronic myeloid leukemic cells in culture causing frank strand breaks in DNA. Anthrapyrazoles, which are also potential photodynamic agents, become strong oxidizers upon illumination with visible light. UV-irradiation of nitrite anion under anerobic conditions generates both the hydroxyl radical ($^{\bullet}OH$) and nitric oxide (NO). In the presence of organic substrates the hydroxyl radical gives rise to secondary carbon radicals which can react with oxygen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50080-10 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Environmental Health Applications of Mass Spectrometry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
OTHER:	Carol E. Parker	Chemist	LMB	NIEHS
	Steven McGown	Chemist	LMB	NIEHS
	Leesa Deterding	Chemist	LMB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

.55

PROFESSIONAL:

.10

OTHER:

.45

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

One of the components of the mass spectrometry workgroup's mission is to provide other groups within NIEHS access to mass spectrometric analyses on a service basis. The workgroup provides the following services on an ongoing basis: 1) low and high resolution electron impact (EI) mass spectra; 2) low and high resolution chemicals ionization (CI) mass spectra; 3) negative ion chemical ionization (NICI) mass spectra; 4) gas chromatography/mass spectrometry (GS/MS) in conjunction with EI, CI, and NICI MS; 5) thermospray (TSP) liquid-chromatography/mass spectrometry (LC/MS) in conjunction with CI and NICI MS; 6) electrospray ionization (ESI) MS coupled with capillary electrophoresis and nanoscale packed LC; 7) fast atom bombardment (FAB) under both positive and negative ion conditions; 8) continuous flow FAB/MS and FAB/MS/MS under both positive and negative ion conditions; and 9) tandem MS in combination with positive and negative ion FAB, ESI, EI and CI MS. We also provide high performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE) support.

During the past year approximately 900 MS analyses have been performed on a service basis (not including collaborative work).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 ES 50087-06 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Singlet Oxygen-Dependent Photosensitivity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute)

PI:	Resza Dabestani	Senior Staff Fellow	LMB	NIEHS
	Colin F. Chignell	Chief, LMB	LMB	NIEHS
	Krzysztof Reszka	Visiting Scientist	LMB	NIEHS
OTHER:	Robert H. Sik	Biologist	LMB	NIEHS

COOPERATING UNITS (if any)

Dr. Ann G. Motten, Duke University, Durham, North Carolina

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Photobiology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Photosensitization can result when light interacts with endogenous or exogenous chemical agents in the skin and eyes. This process can produce undesirable clinical consequences, as in phototoxicity and photoallergy; or it can have beneficial effects, as in tumor photodynamic therapy (PDT) and coal-tar or psoralen (PUVA) therapy against psoriasis. Photosensitization results from the light-induced production of free radicals and/or singlet oxygen ($^1O_2$), the lowest electronic excited state of molecular oxygen. Because the latter species may be important in both phototoxic reactions and PDT, we have developed state-of-the-art instrumentation capable of detecting the characteristic phosphorescence of $^1O_2$ at 1268 nm. This instrumentation has permitted us to delineate the photophysics of $^1O_2$ production from a number of photosensitizers including phenothiazines, tetracycline, benzoxazoles synthetic dyes, anthralin and 1,8-dihydroxy-anthraquinone. Singlet oxygen has been implicated in the phototoxicity of benzanthrone (7H-benz[de]anthracene-7-one), a dye intermediate prepared from 1,8-dihydroxyanthraquinone. Potential photodynamic agent 1,5-diamino-4,8-dimethoxy-anthraquinone and related compounds were shown to be efficient $^1O_2$ generators which may explain their cytotoxicity to human leukemic cells in culture. The influence of cationic surfactants on the photoprocesses of the sensitizers eosin and rose bengal have been studied in both aqueous and organic solvents. A nano-second laser flash photolysis spectrometer has been built and successfully tested. This equipment now permits us to carry out time-resolved transient absorption and emission spectroscopy on excited state intermediates (precursors to $^1O_2$) of photosensitizers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50088-06 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Relationship of Free Radicals to Halocarbon-Induced Toxicity in the Liver

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	Ronald P. Mason	Research Chemist	LMB	NIEHS
OTHER:	Kathy T. Knecht	Guest Worker	LMB	NIEHS
	Janice A. DeGray	IRTA Fellow	LMB	NIEHS

COOPERATING UNITS (if any)

Dr. Ronald G. Thurman, Department of Pharmacology, UNC, Chapel Hill, North Carolina

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Electron Spin Resonance

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The volatile anesthetic halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) causes liver damage in humans. It has been suggested that free radicals produced by the reduction of halothane may not only bind to cellular constituents but may also induce lipid peroxidation. Indeed, others have reported the spin trapping of lipid-derived radicals after halothane treatment *in vitro* and *in vivo*. Presently, two radical adduct species have been detected in the bile of living rats treated with halothane and PBN. The treatment of rats with hypoxia (12% oxygen) was required for radical adduct detection, consistent with a known requirement for hypoxia in experimental hepatotoxicity in phenobarbital-retreated rats. Isotopic studies conclusively demonstrated that both radical adduct species arose from the reductive debromination of halothane and not from lipid peroxidation, as others had previously reported.

Free radical formation from ethanol *in vivo* has been described earlier. *In vivo* studies show that free radical formation from ethanol in a microsomal system is dependent upon contaminating trace metal and the production of superoxide anion, rather than upon enzymatic activity of cytochrome P-450, as reported in the literature. Thus, these studies call into question the significance of a microsomal pathway for free radical formation. Further work is being performed to determine the source of ethanol-derived free radicals *in vivo* and their role in toxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50106-04 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Collaborative Projects in Environmental Health Sciences

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
OTHER:	Carol E. Parker	Chemist	LMB	NIEHS
	Leesa Deterding	Chemist	LMB	NIEHS
	Steven McGown	Chemist	LMB	NIEHS
	Hideo Iwahashi	Visiting Scientist	LMB	NIEHS
	Ronald Mason	Research Chemist	LMB	NIEHS
	Daniel Morgan	Research Chemist	STB	NIEHS

COOPERATING UNITS (if any)

Prof. J. Frelinger, Dept. Microbiology & Immunology, UNC, Chapel Hill, NC; Dr. Lynda Tussey, Molecular Immunology, Oxford Univ. UK; Dr. Michalopoulos, Dept. Pathology, and Dr. W. Storkus, Molecular Genetics, Univ. Pittsburgh.

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.05

PROFESSIONAL:

.65

OTHER:

.40

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Collaborative projects in the environmental health sciences includes those projects in which the mass spectrometry work-group collaborates with other groups, both within and without the institute to solve problems of mutual interest. The major focuses of these projects are 1) the structure determination of biological compounds of unknown structure, 2) identification and/or confirmation of biological pathways and 3) development of strategies for the structure determination of biologically important compounds.

Major projects currently under investigation include; 1) the structure determination of hepatopoietin B, a low molecular weight compound in serum that stimulates hepatocyte DNA synthesis and is hypothesized to be the prototype molecule mimicked by xenobiotic compounds, such as phenobarbital, that cause hepatocyte hypertrophy and proliferation; 2) the structure determination of the small peptides expressed by cells infected with flu virus that cause the cell to be recognized by T-cells as being infected (a model of HIV infection) and of the small peptides expressed by cancer cells; 3) the development of combined LC/EPR/MS techniques for the identification of spin-trapped free radicals. This latter project involves the structure determination of chemically produced free-radicals and of free-radicals produced as intermediates in enzymatic processes in biological systems. 4) Determination of blood levels of styrene and styrene oxide in rats exposed to styrene by injection or inhalation. We are also developing techniques that will allow us to examine hemoglobin from the exposed rats for hemoglobin adducts of styrene oxide to be used as a marker for exposure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50107-04 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Nanoliter Capillary LC/MS Techniques

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
Other:	Leesa Deterding	Chemist	LMB	NIEHS
	Steven McGown	Chemist	LMB	NIEHS
	John Perkins	Visiting Fellow	LMB	NIEHS
	Franktisek Pehal	Visiting Associate	LMB	NIEHS
	Carol Parker	Chemist	LMB	NIEHS

COOPERATING UNITS (if any)

Prof. D. Hunt, University Virginia; Prof. Y. Shido, K. O'Hara, M. Kono, Tokyo College of Pharmacy, Japan; Prof. M.L. Gross and Dr. R.L. Cerny, Univ. Nebraska

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS; NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

3.0

2.1

.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A perennial problem in the mass spectrometric analysis of both biological and environmental samples is that the absolute level of analyte is extremely low. One approach to this problem is to develop low volume-high flux delivery systems for the mass spectrometer. We have undertaken the development of interfaces for nanoscale packed capillary LC and MS. These capillary systems offer the same advantages over wider-bore LC systems that capillary GC offers over packed-column GC, a high flux of analyte into the MS but with a significantly lower total analyte level necessary. Current developments are in the areas of nanoscale capillary LC and capillary zone electrophoresis interfaced with both coaxial continuous flow fast atom bombardment MS and electrospray ionization MS, including electrospray ionization on a magnetic sector instrument. We have applied these techniques to separations of, for example, sulfonamides, quinolinic acid antibiotics, macrol antibiotics, penicillins, peptides and complex mixtures of proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50108-04 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Tandem Mass Spectrometry for Structure Elucidation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Kenneth Tomer Research Chemist LMB NIEHS

OTHER: Leesa Deterding Chemist LMB NIEHS
Steven McGown Chemist LMB NIEHS
John Perkins Visiting Fellow LMB NIEHS
John Curtis Chemist LMB NIEHS

COOPERATING UNITS (if any)

Dr. L.Q. Huang, Connecticut Agricultural Experimentation Center; Prof. M.L. Gross, Dr. R.L. Cerny, University Nebraska; Professors Y. Shida, K. O'Hara, M. Kono, Tokyo College of Pharmacy; Prof. J. Frelinger, UNC Dept. Microbiology/Immunology;

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

9

25

65

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A major program in the mass spectrometry laboratory at NIEHS is the application of tandem mass spectrometric techniques (MS/MS) to the structure elucidation of compounds of interest in the environmental health sciences. The structure determination of these compounds is basic to understanding the interactions of compounds within the body, especially those due to altered metabolism and those arising through the interactions of xenobiotics and biomolecules. These techniques are important because samples of interest are often complex mixtures and because the ionization techniques applicable to these samples often provide little or no structural information.

Our approach to the development of MS/MS techniques is twofold: structure elucidation and increasing the sensitivity of the technique. Current projects in the area of structure determination include: 1) determination of the location of substituents and double bonds in compounds within the arachidonic acid cascade under negative ion FAB/MS/MS conditions; 2) development of hybrid MS/MS techniques in combination with electrospray ionization; 3) determination of the structure of macrolide antibiotics and the bacterial enzymatic deactivation products; 4) structure determination of peptides expressed by flu-infected cells (biological model systems for AIDS infected cell recognition); 5) application of MS/MS to the structure determination of aminoglycoside antibiotics. A major effort in increasing MS/MS sensitivity has been in the combination of high flux/low level introductory systems such as nanoscale packed LC and CZE. We have successfully lowered the acquisition levels for a acquisition levels 50 nm number of analyte types including protein digests, peptides, modified nucleosides, HETEs, and leukotrienes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50109-04 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oxygen-Centered Free Radical Formation by Peroxidases and Lipoxygenase

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Ronald P. Mason Research Chemist LMB NIEHS

OTHER: Walee Chamulitrat Visiting Associate LMB NIEHS
Phillip M. Hanna Postdoctoral IRTA LMB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Electron Spin Resonance

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lipoxygenase is an enzyme which catalyzes the oxidation of unsaturated fatty acids to hydroperoxides. The oxidation of fatty acids by 5-lipoxygenase is the first step in leukotriene biosynthesis, which has been implicated as a mediator in inflammation and hypersensitivity reactions. Therefore, the inhibition mechanism of lipoxygenase and the development of clinically useful lipoxygenase inhibitors have become the subject of intensive research. One proposed mechanism of the inactivation of lipoxygenase by inhibitors is the reduction of the catalytically active ferric form of the enzyme to its ferrous form. Recent studies have shown that compounds containing the hydroxamate moiety are potent inhibitor is thought to bind to iron at the catalytic site of the enzyme. We now report evidence that the NOH of the hydroxamate group of N-(4-chlorophenyl)-N'-(3-chlorophenyl) urea (CPHU), N-[(E)-3-(3-phenoxyphenyl)prop-2-enyl]aceto-hydroxamic acid (BW A4C), and N-(1-benzo(b)thien-2-ylethyl)-N-hydroxyurea (Zileuton) is oxidized by lipoxygenase to form their corresponding nitroxides, which are directly detected by electron paramagnetic resonance spectroscopy. It is consistently found that the selected NOH-containing compounds, e.g., alkylhydroxylamines or N-hydroxyureas, are also oxidized by lipoxygenase to form their corresponding nitroxides.

Studies involving a nucleophilic addition by water to a nitron spin trap, DMPO, were extensively investigated. The HPLC/EPR results demonstrated that the assignment made by previous workers was not correct due to this nucleophilic reaction which partially contributed to the DMPO radical adduct.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50110-04 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NMR Studies of Cellular Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Robert E. London Research Physicist LMB NIEHS

OTHER: Louis A. Levy Chemist LMB NIEHS
Bruce Berkowitz Senior Staff Fellow LMB NIEHS
Tersa Fralix Staff Fellow LMB NIEHS

COOPERATING UNITS (if any)

Dr. Robert Chapin, STB, DTRT and Dr. Warren Ku, STB, DTRT

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Nuclear Magnetic Resonance Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.1

PROFESSIONAL:

0.7

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This program is aimed at the development and application of *in vivo* NMR spectroscopic methods for studying metabolism and its perturbation by chemical toxins. The two primary applications during the past year were: (1) measurements or oxygen levels in the vitreous compartment of the rabbit eye, and its perturbation by various agents, and (2) studies of the effects of boric acid on the carbohydrate metabolism of Sertoli cells. In the first series of studies, oxygen levels have been determined based on the concentration-dependent perturbation of the spin lattice relaxation rate of perfluorotributyl amine. This approach requires that either all or a portion of the vitreous humor be replaced by the perfluorocarbon. Two types of measurement were carried out, one in which the vitreous was entirely replaced, and a second in which small, 5 um spheres of perfluorocarbon were placed in the eye. Effects of damage to the blood-retinal barrier on the oxygen level of the vitreous were quantified, and a protective effect of steroids was demonstrated.

A second series of studies is aimed at understanding the physiological effects of boric and boronic acids, and the potential basis for the toxic effects of these compounds which have been reported. Although boron is an essential element for plants, no need in animal systems has been established. Recently it has been demonstrated that at relatively high levels boric acid is selectively toxic to the male reproductive system. NMR studies of the effects of boric acid on the metabolism of sertoli cell cultures have been carried out using carbon-13 labeled glucose. An initial series of measurements indicates that the glutamine/glutamate ratio is significantly reduced in cells exposed to boric acid.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 50111-04 LMB	
PERIOD COVERED October 1, 1991 to September 30, 1992			
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>NMR Studies of Biomolecular Structure, Function, and Dynamics</u>			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)			
PI:	Robert E. London	Research Physicist	LMB NIEHS
Other:	Donald G. Davis	Expert	LMB NIEHS
	Michael E. Perlman	Senior Staff Fellow	LMB NIEHS
COOPERATING UNITS (if any) Dr. M. Thompson, DTRT, Drs. G. W. Koszalka, and Thomas A. Krenitsky, Burroughs Wellcome, Research Triangle Park, NC			
LAB/BRANCH Laboratory of Molecular Biophysics			
SECTION Nuclear Magnetic Resonance			
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709			
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:	
3.4	2.2	1.2	
CHECK APPROPRIATE BOX(ES) ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews			
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The aims of this project are: (1) to develop and modify NMR methodologies for the assignment of resonances and the analysis of the structure and dynamics of molecules in solution, and (2) to apply these methods to problems of concern in relation to environmental health. During the past year, the primary emphasis of this project has been in two areas: (1) development of the transferred NOE (TRNOE) technique and its application to the determination of the conformation of the nucleoside drug tubercidin in the complex with <i>E. coli</i> purine nucleoside phosphorylase (PNPase), and (2) analysis of the interaction of benzenboronic acid (BBA) derivatives with small molecules and with the enzyme subtilisin. An important objective of both problems was a characterization of the kinetic behavior. Specifically, we have previously determined that the TRNOE parameters for inhibitors which are exchanging with macromolecular binding sites can be strongly dependent on the exchange rates. We have recently evaluated the use of two strategies for determining ligand exchange rates: (1) Measurements of T_2 using the CPMG technique as a function of pulse rate, and (2) measurement of the T_1 values for the ligand as a function of the strength of the spin-lock field. We are currently carrying out detailed theoretical and experimental analyses of these experiments in the PNPase - tubercidin system. In the second area, studies with BBA derivatives have determined apparent dissociation constants with various biologically significant ligands under physiological conditions, as well as binding kinetics. The binding kinetics of 4-fluorobBA with the serine protease subtilisin were compared with results obtained on various model ligands in order to characterize the interaction with the enzyme.			

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 50112-04 LMB
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Magnetic Resonance Imaging Studies of Heavy Metal Distribution		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)		
PI:	Robert E. London Research Physicist LMB	NIEHS
OTHER:	Bruce Berkowitz Senior Staff Fellow LMB	NIEHS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Molecular Biophysics		
SECTION Nuclear Magnetic Resonance		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3
CHECK APPROPRIATE BOX(ES) ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Over the past decade, magnetic resonance imaging (MRI) has become an important medical diagnostic tool. The contrast in these images is a function of the density of proton spins in the volume of interest, and of the relaxation properties of these spins. Chemical agents which alter these relaxation properties can alter the observed contrast, and hence the distribution of these "contrast agents" has become a useful diagnostic tool. Two types of agents can be used for such purposes: chelated paramagnetic ions, now in widespread clinical use, and stable organic radicals. During the past year we have investigated the factors which govern the distribution of these agents in the rat brain. Agents were introduced intracerebrally in order to evaluate the potential diagnostic utility of this route of administration. It was found that positively charged agents including bare metal ions as well as a chelated Gadolinium-HAM complex were strongly associated with the walls of the cerebral ventricles. However, although manganese ions administered intraperitoneally rapidly crossed the blood brain barrier, the observed contrast did not indicate association with the ventricle walls. This difference indicates that manganese ions administered ip behave more like chelated ions, and are most probably complexed to transferrin. These studies have recently been extended to comparisons of the effects of Gd-DTPA with those of several stable organic radicals. Comparison of the contrast effects allows evaluation of the transmembrane flux of the organic radicals.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50114-04 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Xenobiotic Metabolism in Lower Species

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Phillip W. Albro

Research Chemist

LMB

NIHES

COOPERATING UNITS (if any)

Comparative Medicine Branch

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Metabolism

INSTITUTE AND LOCATION

NIHES, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.1

PROFESSIONAL:

0.8

OTHER:

1.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has two objectives: (1) To explore the metabolic capabilities of invertebrate species, with emphasis on the ability to metabolize common environmental pollutants. Initially we are studying compounds whose metabolism is well understood in mammals, in order to make comparisons. (2) To investigate the possibility that some types of metabolism studies, especially those which must be performed *in vivo*, can be effectively accomplished in species having less developed nervous systems (and are thus presumably less subject to pain and distress) than the more commonly used rodent species. We are presently studying *Lumbricus terrestris*, the common earthworm ("night crawler") because it has been relatively neglected in studies of metabolic capabilities, and because it is typically exposed to environmental pollutants in landfills.

We have established that earthworms, in symbiosis with a single species of intestinal bacteria, metabolize phthalate plasticizers completely to carbon dioxide. This may be an ideal model in which to study cooperative metabolism, since the biological system can be simplified to just two species.

Earthworms appear to have very limited ability to regulate the lipid composition of their tissues. It is possible to modify the lipid composition extensively by dietary control, which may be useful in future studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50115-04 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Computerized Spin Trapping Data Base

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Colin F. Chignell Chief LMB NIEHS
Anson S.W. Li Staff Specialist LMB NIEHS

COOPERATING UNITS (if any)

Dr. Garry R. Buettner, University of Iowa, HBRF 68, Iowa City, IA

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Photobiology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Spin trapping is a powerful and convenient technique for the study of free radical reactions. The breadth of applications ranges from clinical studies to high-energy physics. Over 1500 references to the technique have accumulated in Chemical Abstracts. STDBII, a spin trapping database, has been implemented on IBM PC/AT and Macintosh personal computers. The package operates with no "add-ons." The program is powerful yet user-friendly; the command structure is similar to the familiar 1-2-3 light-bar menu; search strategy employs the method of Query-by-Example (QBE); logical combinations of any fields are accomplished by using AND, OR, NOR, and EXCEPT. Presently, STDBII (4.5) contains files for 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), alpha-phenyl-N-tert-butyl nitron (PBN), 2-methyl-2-nitrosopropane (MNP), alpha-(4-pyridyl-1-oxide)-N-tert-butyl nitron (POBN), nitrosodurene (ND) and 3,5-dibromo-nitrosobenzene sulfonate (DBNBS). Data for other less popular traps are included in a catch-all file. Presently, the database files have more than 2000 references with over 8800 parameter entries. The STDBII files contain information on: 1) spin trap used; 2) radical trapped; 3) hyperfine splittings reported; 4) solvent; 5) g-value, if reported; 6) a terse description of how the radical was produced and observed; 7) full bibliographic data. The latest version (STDBII, 4.5) also contains kinetic data on spin trapping rates as well as a new parameter, the NoH value (the ratio of a_N/a_H), which has proved useful for the identification of DMPO adducts. STDBII helps researchers: 1) in identification of spin adducts from the sometimes unique hyperfine splitting parameters; 2) as a key to the spin trapping literature 3) as a vehicle to correct published errors. STDBII is now available to researchers both inside and outside NIEHS. The package includes a user manual that lists all of the compiled information on spin trapping. Scientists who do not presently have access to a computer can still benefit from STDBII because all of the database entries are printed in the STDBII User Manual.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50116-03 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Microdialysis/Mass Spectrometry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIHES
OTHER:	Leesa Deterding	Chemist	LMB	NIHES
	Leo T. Burka	Research Chemist	LMB	NIHES
	Kelly Dix	Guest Researcher	STB	NIHES

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIHES, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

.5

.1

.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Microdialysis is a relatively new sampling technique designed for the *in vivo* analysis of endobiotic/xenobiotic substances. The microdialysis probe is surgically implanted into a vein or a tissue of interest in an animal. Substances within the bloodstream or tissue with M_r below the molecular weight cutoff of the dialysis membrane dialyze across the membrane into the perfusate. We have interfaced this technique with mass spectral analysis using the coaxial continuous flow fast atom bombardment (CF-FAB) approach we have developed and tandem mass spectrometry.

Our initial experiments have concentrated on the investigation of the half-life of the suspected carcinogen *tris* (2-chloroethyl)-phosphate in the blood of rats. *Tris* organophosphates have been widely used in plastics and synthetic fibers as flame retardants. We have determined the half-life of this compound in rats (introduced by injection into the blood stream) using both microdialysis/MS in anesthetized rats and in anesthetized and awake rats using conventional pharmacokinetic techniques. The free plasma concentration vs. time followed a one compartment model using the program NONLIN84. There were definite statistical differences in the results obtained by the different methodologies. The microdialysis/MS results were similar to those reported for microdialysis/LC determinations of theophylline in anesthetized rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 50117-02 LMB	
PERIOD COVERED October 1, 1991 to September 30 1992			
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Transition-Metal Mediated Free Radical Formation In Vitro and In Vivo</u>			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)			
PI:	Ronald P. Mason	Research Chemist	LMB NIEHS
Other:	Phillip Hanna	IRTA	LMB NIEHS
	Maria Kadiiska	Visiting Scientist	LMB NIEHS
	David Duling	Programmer/Analyst	CSC NIEHS
	Sandra Jordan	Biologist	LMB NIEHS
COOPERATING UNITS (if any)			
LAB/BRANCH Laboratory of Molecular Biophysics			
SECTION Electron Spin Resonance			
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709			
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:	
3.0	2.0	1.0	
CHECK APPROPRIATE BOX(ES) ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews			
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Although free radical formation is known to occur from copper in Fenton-like reactions <i>in vitro</i>, free radicals from copper have not been detected in living animals until now. By applying ESR and the secondary radical spin trapping technique, evidence for hydroxyl radical formation <i>in vivo</i> was obtained in rats treated simultaneously with copper and ascorbic acid. The existence of hydroxyl radical has also been shown in the bile of selenium- and vitamin E- deficient rats during conditions of acute copper overload. We demonstrate that hydroxyl radical generated by copper can indeed occur <i>in vivo</i> and contribute to oxidative damage, though in our model of copper-intoxicated rats copper alone does not cause the formation of detectable radical adducts. Paraquat is a toxin whose toxicity is attributed to its ability to generate the superoxide radical anion. Increased superoxide production can in turn stimulate hydroxyl radical formation <i>in vitro</i> and <i>in vivo</i> from metal ions. We have shown this to be true for both copper and iron overload experiments in rats. Some metal ions may also have beneficial effects on free radical metabolites <i>in vivo</i>. Our results show a suppression of free radical metabolites detected in rats from the metabolism of the hepatotoxin carbon tetrachloride due to pretreatment with trivalent chromium. The formation of artifactual radical adducts in spin trapping experiments is a possibility that must always be guarded against. We have demonstrated experimental procedures necessary to inhibit <i>ex vivo</i> metal ion-induced free radical chemistry in cases of metal overload. This is crucial for correctly identifying radicals generated <i>in vivo</i>. We have established certain criteria for suppressing artifactual ESR signals <i>in vitro</i> resulting from non-radical chemistry of the spin trap DMPO in the presence of copper and iron ions.			

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50118-02 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Mechanism of Reduction of Toxic Chemicals and Drugs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	Ronald P. Mason	Research Chemist	LMB	NIEHS
OTHER:	Janice DeGray	IRTA Fellow	LMB	NIEHS
	Sandra Jordan	Biologist	LMB	NIEHS
	David Duling	Programmer/Analyst	LMB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Electron Spin Resonance

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The first step in the metabolic conversion of many chemicals and toxic drugs is the reduction of the parent compound to give a free radical intermediate. This metabolism has been shown to occur in both liver microsomes and hepatocytes. For nitroheterocyclic drugs, the nitro group is reduced to the corresponding nitro radical anion and for bipyridylum dications, the parent compound is reduced to the bipyridylum radical monocation. By judicious application of paramagnetic line broadening agents, we have shown that the radicals, initially formed inside the cell can freely cross the plasma membrane, and the EPR signal detected is mainly due to radical present extracellularly. Recently, the laboratory purchased a Krumdieck tissue slicer. This type of slicer has been shown to produce reproducibly thin slices with maximal slice integrity. Liver slices, unlike microsomes or hepatocytes, contain a cross section of all liver cells and the results are more physiologically similar to *in vivo* liver metabolism. This is the first such EPR application to organ slices which have not been lyophilized or encased in paraffin supports. Preliminary experiments with bipyridylum dictations have shown that it is possible to detect free radical intermediates in thin liver slices and that the radical generated is in a more viscous environment than with either microsomes or hepatocytes. In the coming year, we will continue this line of investigation and extend the experiments to include nitroheterocyclic compounds.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50119-01 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Properties and Functions of the Melanin Skin Pigment

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	Krzysztof Reszka	Visiting Scientist	LMB	NIEHS
OTHER:	Colin F. Chignell	Chief, LMB	LMB	NIEHS

COOPERATING UNITS (if any)

Dr. Kowichi Jimbow, Division of Dermatology and Cutaneous Sciences, University of Alberta, Edmonton, Alberta, Canada

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Photobiology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.4

PROFESSIONAL:

0.4

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Melanin is the natural pigment synthesized in highly specialized cells, melanocytes, present in the skin. The primary function of the endogenous melanin is photoprotection of skin and eye tissues against the harmful action of UV light. Overproduction of the melanin pigment is characteristic of melanoma, a form of skin tumor. This research project has three main objectives: to study molecular mechanisms of the melanocytotoxic action of phenolic thioether amines (PTEA); to study free radical processes induced by UV irradiation of pheo- and eumelanin pigments and melanogenic compounds cysteinyl dopas and hydroxyindoles; to study electron-transfer properties of melanins and melanin precursors.

We found that incubation of depigmenting PTEA agents 4-S-cysteinylphenol (4-S-CP), 4-S-cysteaminyphenol (4-S-CAP), 4-S-homo-cysteaminyphenol (4-S-homo-CAP) and N-acetyl-4-S-cysteaminy phenol (N-Acetyl-4-S-CAP) in aerated aqueous buffer (pH 6) in the presence of mushroom tyrosinase and metal ions (Mg^{2+} , Zn^{2+}) produced ortho-semiquinone radical/metal ion complexes. In the absence of the metal ions the formation of free radicals was observed only from 4-S-CP and 4-S-CAP. Other PTEA agents afforded weak, melanin-like EPR signals. UV photolysis (>300 nm) of by-products of melanin biosynthesis 5-hydroxy-6-methoxyindole-2-carboxylic acid and 5-methoxy-6-hydroxyindole-2-carboxylic acid in aqueous solutions (pH 10) generated indoloxyl free radicals, hydrogen atoms and/or hydrated electrons. The indoloxyl radicals are quenched by ascorbate to produce the ascorbyl radical. The electron transfer capacity of synthetic DOPA-melanin and melanin precursors (tyrosine, phenol, DOPA, catechol, 6-hydroxy-dopamine) was tested using 4-methoxy-benzenediazonium tetrafluoroborate as an electron acceptor. Melanin, catechol, DOPA and 6-hydroxy-dopamine efficiently reduced the diazonium compound which resulted in the formation of the highly reactive 4-methoxy-phenyl radical. Tyrosine and phenol reduced the diazonium compound only upon activation by the enzyme tyrosinase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50120-01 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Tumor-Promoting and Anti-Psoriatic Mechanisms of 9-Anthrone

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	Patrick Hayden	PRAT Position	LMB	NIEHS
OTHER:	Colin F. Chignell	Chief, LMB	LMB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Photobiology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.6

PROFESSIONAL:

1.1

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Certain 1,8-dihydroxy-9-anthrone have been used for the topical treatment of psoriasis. The therapeutic usefulness of these compounds is limited, however, by side effects including severe skin irritation and staining. Some anti-psoriatic anthrones are also tumor-promoters. The chemical mechanisms underlying the biological properties of 9-anthrone are uncertain, but are believed to be dependent upon the generation of free radical products such as 9-anthrone-10-yl radicals or secondary oxygen radicals such as $O_2^{\cdot-}$ or $OH^{\cdot}$. The goal of this project is to gain a better understanding of the chemical and biochemical mechanisms of 1,8-dihydroxy-9-anthrone anti-psoriatic and tumor-promoting activities. Knowledge of these mechanisms will be useful for the design of more effective anti-psoriatic agents and will provide insights into fundamental mechanisms of cell growth and proliferation, which may also help in the understanding of other diseases such as cancer. The free radical species produced by a series of 9-anthrone of known tumor-promoting and anti-psoriatic activities have been investigated. Direct EPR and spin trapping techniques were utilized to detect radical products in aqueous buffers and in a keratinocyte culture system. Anthrone-10-yl radicals were identified as spin adducts of 2,6-dibromonitrosobenzene sulfonate (DBNBS). The ability to form these adducts was pH dependent and appears to be an important property of anti-psoriatic anthrones. A radical species was also detected by direct EPR in human keratinocytes after treatment with some biologically active anthrones. The formation of this species was shown to be light dependent. 5,5-Dimethyl-pyrroline-N-oxide (DMPO) was used to trap reactive oxygen radicals. Because all of the 9-anthrone tested generated significant amounts of DMPO spin adducts irrespective of their anti-psoriatic or tumor-promoting activities, we conclude that the ability to generate oxygen radicals is not sufficient to provide these activities, but may be involved in skin irritation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 50121-01 LMB
PERIOD COVERED <u>October 1, 1991 to September 30, 1992</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Development of Intracellular Indicators and Ion Transport Studies</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute)		
PI:	Robert E. London	Research Physicist LMB NIEHS
OTHER:	Elizabeth Murphy	Research Physicist LMB NIEHS
	Louis A. Levy	Chemist LMB NIEHS
	Chung Rhee	Visiting Fellow LMB NIEHS
COOPERATING UNITS (if any) Professor Melvyn Lieberman, Division of Physiology, Department of Cell Biology, Duke University Medical Center, Durham, N.C.		
LAB/BRANCH <u>Laboratory of Molecular Biophysics</u>		
SECTION <u>Nuclear Magnetic Resonance</u>		
INSTITUTE AND LOCATION <u>NIEHS, NIH, Research Triangle Park, North Carolina 27709</u>		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.1	1.4	0.7
CHECK APPROPRIATE BOX(ES) ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) It is well recognized that ions can function as intracellular messengers. It is not surprising, therefore, that alterations in ion transport frequently cause cellular dysfunction. For example, many oncogene products alter ion transport. The goal of this project is twofold: (1) development of new indicators for the measurement of intracellular ion concentrations, and (2) investigation of ion transport and its regulation, and the perturbations which accompany pathogenic states. Previously we have developed new NMR-active indicators for cytosolic calcium, magnesium, and chloride, and for cell volume. These NMR indicators are generally (although not necessarily) fluorinated molecules since fluorine-19 is 100% abundant, highly sensitive for NMR detection, and since cells are essentially free of any background fluorine NMR signals. Two major projects over the past year were: (1) development of a new, high dissociation constant (K_d) intracellular calcium ion indicator, "TFBAPTA", and (2) synthesis and evaluation of several derivatives of "NEAP", our recently developed pH sensitive indicator. One of the principle limitations on the use of cytosolic calcium ion indicators in the perfused heart has been the significant perturbation of contractility which results from the introduction of intracellular calcium ion chelators. This was the primary motivation for our development of a new indicator, TFBAPTA, which has a considerably higher calcium ion dissociation constant and is therefore essentially non-perturbing. Although not suited for the determination of basal calcium ion concentrations, this indicator is useful for measuring the increases which accompany various perturbations such as ischemia or the administration of certain chemical agents. We have also made several further modifications of the pH indicator NEAP in order to reduce leakage and to add an internal reference signal in order to facilitate quantitation. WE are also continuing studies on the transport mechanisms that regulate cytosolic free magnesium well below its electrochemical equilibrium.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50122-01 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Linoleic Acid Metabolism in Modulating Oncogenic Transformation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Wayne C. Glasgow Senior Staff Fellow LMB NIEHS

OTHER: Angela E. Everhart Biologist LMB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Eicosanoid Biochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The polypeptide mitogen Epidermal Growth Factor (EGF) stimulates quiescent cell cultures to initiate DNA synthesis and cell division. Binding of EGF to specific cell surface receptors activates a variety of biochemical pathways. The role of each of these biochemical processes in transducing the mitogenic signal has yet to be clearly delineated. One biochemical pathway implicated in modulating the EGF response is linoleic acid metabolism. Our laboratory has reported that specific lipoxygenase-derived metabolites of linoleic acid regulate growth factor signal transduction in fibroblasts. We now wish to examine whether linoleic acid metabolism can also play a role in the uncontrolled proliferation characteristic of neoplastic cells. We have transformed normal fibroblasts with either *v-erbB* oncogene or its cellular homolog, *c-erbB2*. The *erbB* oncogene of avian erythroblastosis retrovirus encodes a truncated EGF receptor and transforms cells via functioning as an activated growth receptor. We propose to study the effects of *erbB* oncogene expression on linoleic acid metabolism, and to compare these responses to the normal EGF-stimulated activity. The studies include characterizing the linoleic metabolites found in transformed cells, testing specific metabolites for their ability to modulate oncogene signal transduction, and examining the effects of blocking linoleic acid metabolism on altering the transformation process. These investigations should provide important information regarding the underlying mechanisms of normal growth control and neoplasia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50123-01 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Growth-Factor Stimulation of Lipoxygenase Oxidation of Biological Membranes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Wayne C. Glasgow Senior Staff Fellow LMB NIEHS

OTHER: Angela E. Everhart Biologist LMB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Eicosanoid Biochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.50

PROFESSIONAL:

0.25

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The oxygenation of free polyenoic fatty acids via the lipoxygenase pathway leads to the formation of potent mediators such as leukotrienes which are involved in a variety of physiological and pathological processes. In addition to the free fatty acid substrates, a number of 15-lipoxygenases catalyze the specific oxygenation of phospholipids. Moreover, many lipoxygenase products are rapidly incorporated into phospholipid and triglyceride of intracellular membranes. This lipoxygenase-stimulated oxygenation of membranes is a critical event in the transition of reticulocytes to erythrocytes. These findings have prompted us to study the effects of epidermal growth factor (EGF) activated lipoxygenase metabolism of fibroblast membranes. We have previously found that EGF induces the biosynthesis of lipoxygenase-derived free acid products in fibroblasts; and these compounds are an important component of the mitogenic signal. Analytical chemical characterization of the phospholipid fraction of these fibroblasts revealed that EGF treatment resulted in a significant increase in oxidized esterified products. The primary membrane product was identified as 13(S)-hydroxy-octadecadienoic acid. Further studies will address the mechanism of EGF-stimulated membrane oxygenation. We will determine whether these products arise from direct lipoxygenase metabolism of phospholipid or via re-acylation reactions. We will also examine the importance of esterified products in modulating EGF-dependent fibroblast mitogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80008-17 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biosynthesis of Prostaglandins, Hydroxy-Fatty Acids and Leukotrienes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	Thomas E. Eling	Research Chemist	LMB	NIEHS
OTHER:	Wayne Glasgow	Staff Fellow	LMB	NIEHS
	Julie Angerman-Stewart	Biologist	LMB	NIEHS
	Rebecca Hardman	Biologist	LMB	NIEHS
	Angela	Biologist	LMB	NIEHS
	Matthew Cowlen	IRTA Fellow	LMB	NIEHS
	Jeffrey Kitzler	Staff Fellow	LMB	NIEHS
	Elizabeth Hill	Visiting Associate	LMB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Eicosanoid Biochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

5.25

PROFESSIONAL:

3.25

OTHER:

2.00

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Investigations are concerned with the oxidation of arachidonic and linoleic acids to prostaglandin (PG), leukotrienes and hydroxy-fatty acids and the relationship of this metabolism to the regulation or modulation of biological processes. We have studied the role of arachidonic and linoleic acids metabolism in the response of cells to growth factors.

Epidermal growth factor(EGF) activates a unique 15-lipoxygenase that convert linoleic acid to 13-(S)-HODE. The activation of this lipoxygenase is dependent on the tyrosine kinase activity of the EGF receptor which activates the lipoxygenase and appears to enhance its *de novo* synthesis. Inhibition of 13-HODE formation inhibits EGF mitogenesis and the addition to cells greatly enhances the response to EGF. The importance of this 15-lipoxygenase in EGF dependent mitogenesis is established in Syrian hamster embryo cells (SHE) cells and more recently in Balb MK keratinocytes. Studies are currently underway to further investigate the mechanism for the activation of the 15-lipoxygenase by EGF using both biochemical and molecular biology techniques.

We have also studied the regulation of PHS expression in cells. 12-O-tetra-decanoylphorbol-13-acetate (TPA) and other growth factors induce the synthesis of a new PHS by enhancing the expression of PHS m-RNA. Mitogen induces the formation of a new form of PHS that has a unique substrate specificity. Other data indicate that steroids inhibit the expression of PHS. These findings suggest a possible important role for arachidonic and linoleic acid metabolism in regulation cell growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80035-17 LMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cooxidation of Xenobiotics by the Prostaglandin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	Thomas E. Eling	Research Chemist	LMB	NIEHS
OTHER:	Ronald P. Mason	Research Chemist	LMB	NIEHS
	John Curtis	Chemist	LMB	NIEHS
	Jeffrey Kitzler	Staff Fellow	LMB	NIEHS
	Janice DeGray	IRTA Fellow	LMB	NIEHS
	Richard Philpot	Research Chemist	LMB	NIEHS

COOPERATING UNITS (if any)

Dr. Gunter Lassman, Institute Molecular Biology, Berlin, Germany; Dr. Larry Marnett, Dept. of Biochemistry, School of Medicine, Vanderbilt University, Nashville, Tennessee.

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Eicosanoid Biochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.25

PROFESSIONAL:

1.5

OTHER:

.75

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The long range goal of this project is to study the oxidation of chemicals to toxic or carcinogenic metabolites by prostaglandin H synthase (PHS) and to demonstrate the importance of this enzyme system in chemical-induced toxicity or carcinogenesis. We have shown that aromatic amine carcinogens are metabolized to mutagens by PHS. PHS dependent oxidation occurred by a free radical mechanism and resulted in the formation of DNA adducts which can be used as *in vivo* markers for PHS-dependent oxidation. We have further studied the formation of amine mutagens by PHS using bacterial Test System. Amines are first acetylated to the acetlyamine. PHS but not HRP then metabolizes the acetlyamine to nitroacetlyamine. The nitro metabolites are potent direct acting mutagens. This explains the role of bacterial acetylation and the difference between PHS and HRP. Our data suggest that PHS is a versatile enzyme system that can catalyze a variety of reactions which are important in the conversion of chemicals to carcinogenic metabolites in extra hepatic tissue.

We have studied the formation of tyrosyl radicals during the oxygenation of arachidonic acid by prostaglandin H synthase. Several tyrosyl radicals were observed by ESR that were characterized as radicals with two different conformations. These radicals appear to be formed as the result of enzyme catalysis and appear not to be catalytic intermediates. Replacement of the Fe in PHS with Mn gives an enzyme that contains cyclooxygenase activity but no peroxidase activity. Tyrosyl radicals were not observed with this Mn-PHS. Other data suggest that tyrosyl radicals are formed as the result of enzyme inactivation. We are currently attempting to express PHS and generate mutant forms of PHS to more fully characterize the enzymatic mechanism. Also, we have recently detected a mitogen inducible form of PHS that has a different substrate preference than the constitutive PHS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 23000-05 LMC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Alterations During Multistep Carcinogenesis in Mice and Humans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: R.W. Wiseman, Senior Staff Fellow, LMC, NIEHS

Others: C.J. Cochran, Biologist, LMC, NIEHS

P. Soderkvist, Special Volunteer/Visiting Associate, LMC, NIEHS

COOPERATING UNITS (if any)

DIR/LMC: Andy Futreal, Carl Barrett

MIT: Bill Dietrich, Eric Lander

DBRA/LMT: Monika Hegi, Marshall Anderson

Duke Univ.: Jeff Marks, Dirk Iglehardt

DTRT/ETB: Bill Caspary, Bill Maronpot

Univ. of Utah: Mark Skolnick

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Chemical Carcinogenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the past year we have utilized length polymorphisms in simple sequence repeats or "microsatellites" to complete the first allelotyping analyses of murine tumors. Lung and mammary adenocarcinomas of B6C3F1 mice induced by butadiene and methylene chloride were examined. Frequent losses of heterozygosity were observed on chromosome 4 in the lung tumors. The pattern of allelic losses was consistent with a region previously implicated as the site of a tumor suppressor gene by cell hybrid studies. In addition homozygous deletions have been reported in a homologous region of human chromosome 9p for a variety of human tumors including lung carcinomas. The most common allelic losses in mammary tumors were detected on chromosomes 11 and 14 near the p53 and retinoblastoma tumor suppressor genes, respectively. Mutations in the p53 gene were characterized in these tumors by immunological techniques, single strand conformation polymorphism analysis, and direct DNA sequencing. We have also demonstrated the power of microsatellite PCR for human allele loss studies. Losses of heterozygosity in sporadic human breast and ovarian cancers were mapped in detail using a series of microsatellites in the proximal region of 17q near the BRCA1 gene. This gene confers a strong familial predisposition to breast and ovarian cancer. Allele losses were observed in more than 70% of the tumors examined providing strong support for the hypothesis that inactivation of the BRCA1 gene plays a critical role, not only in rare breast and ovarian cancer families, but in very common sporadic tumors as well. As part of this work we developed two new highly informative polymorphisms that lie within the p53 and thyroid hormone receptor alpha (THRA1) genes. Our allele loss studies implicated THRA1 as an excellent candidate for the BRCA1 gene. However, mutation analyses of sporadic breast tumors and German breast cancer families excluded THRA1 as the BRCA1 gene. In collaboration with Dr. Skolnick's group, our current studies are directed at the isolation of BRCA1 by positional cloning techniques. This includes examining additional tumors for allele losses and generating a physical map of a 500 kb region of 17q.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 23001-03 LMC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Human Gynecologic Pathology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: J. Boyd, Senior Staff Fellow, LMC, NIEHS

Others: J. Risinger, Biologist, LMC, NIEHS
H. Nishii, Visiting Fellow, LMC, NIEHS
T. Fujino, Visiting Associate, LMC, NIEHS
F. Liu, Special Volunteer, LMC, NIEHS
K. Collins, Biologist, LMC, NIEHS

COOPERATING UNITS (if any)

Duke Univ.; Montreal General Hospital; Creighton Univ., Lund Univ., Sweden; Univ. of Alabama-Birmingham; Johns Hopkins Univ.; Jikei University, Tokyo; Laboratory of Reproductive and Developmental Toxicology, DIR, NIEHS; M.D. Anderson Cancer Center

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Gene Expression

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

5.0

PROFESSIONAL:

3.0

OTHER

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goals of this project include the analysis of molecular genetic alterations in human uterine pathology, specifically endometrial carcinoma and endometriosis. Additionally, studies are underway to determine the genetic basis of dual primary breast-ovarian carcinoma cases and a possible genetic basis of human gynecologic cancers associated with prenatal exposure to diethylstilbestrol. These studies are being carried out with DNA obtained from archival formalin-fixed, paraffin-embedded tissue blocks, fresh-frozen biopsy samples, and established cell lines in culture. Through the analysis of endometrial hyperplasia and carcinoma samples, we have determined that activating point mutation of codon 12 of the Ki-ras oncogene is an early event in a subset of endometrial carcinomas, whereas mutation of the p53 tumor suppressor gene appears to be a later event in endometrial tumorigenesis. A significant number of endometrial carcinomas exhibit allelic loss and/or mutation of the DCC tumor suppressor gene. Using a cultured human neuronal cell model, the DCC gene was additionally shown to be involved in cellular differentiation. Aberrant expression of transforming growth factor- α as well as a number of additional cytokines (e.g., tumor necrosis factor- β and interleukin-6) was shown to be a common feature of endometrial carcinoma and endometriosis, compared to normal endometrium; a clear role for TGF- α in autocrine growth regulation of endometrial carcinoma cells was demonstrated. Significant progress has also been made in a complete allelotyping analysis of human endometrial carcinoma, and in a partial allelotyping analysis of dual primary breast-ovarian carcinoma, in order to ascertain additional tumor suppressor genes that may be relevant to these cancer types. Finally, studies were initiated to determine a possible molecular genetic basis for diethylstilbestrol-associated human and rodent gynecologic neoplasms; the initial target genes include ras and p53. Future work will be designed to contribute to a complete molecular genetic description of the above described tumor types.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 23002-02 LMC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Cell and Tissue Structure in Negative Growth Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: J.C. Barrett, Research Chemist, LMC, NIEHS
J. Boyd, Senior Staff Fellow, LMC, NIEHS

Others: C. Afshari, predoctoral Fellow, LMC, NIEHS
R. Owen, NRC Fellow, LMC, NIEHS

COOPERATING UNITS (if any)

Chemical Carcinogenesis Group, LMC, DIR, NIEHS (R.W. Wiseman)

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Cellular Carcinogenesis (Barrett) and Gene Expression (J. Boyd)

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The process of development and differentiation are influenced by chemomechanical forces generated by the structural network of the cell, which includes the inter-connected extracellular matrix, cell adhesion molecules, cytoskeleton, and nuclear matrix. Multiple studies support the role of these components in development and differentiation. Alterations in the structural components of the cell often accompany neoplastic progression although the significance of these changes to loss of growth controls in neoplastic cells is poorly understood. We have implicated a tumor suppressor gene product in an intrinsic block of cellular growth responses to growth factor signals under conditions that are cell shape- and attachment-dependent. Cells that have lost a tumor suppressor gene (supB⁻) do not exhibit autonomous anchorage-independent growth but grow in soft agar in the presence of serum and growth factors. Cells that retain tumor suppressing ability (supB⁺) are growth inhibited in agar even in the presence of growth factors. Although nontumorigenic, the supB⁻ cells display alterations of actin microfilament organization characteristic of tumor cells. mRNA and protein levels of the actin binding protein tropomyosin-1 (TM-1) were down-regulated in supB⁻ compared to supB⁺ cells. Expression of an antisense cDNA for hamster TM-1 induced an actin microfilament organization in supB⁻ cells that was indistinguishable from that observed in supB⁺ cells. In addition, supB⁻ clones expressing antisense TM-1 acquired the anchorage-independent growth potential characteristic of supB⁺ clones. These data indicate that TM-1 can regulate microfilament organization and provide direct evidence for cytoskeletal regulation of anchorage-independent growth. Our working hypothesis is that loss of a tumor suppressor gene in the supB⁻ cells results in cell shape-dependent differences in growth and gene expression manifest related to these structural perturbations. The altered cell structure, anchorage-independent growth, and patterns of gene expression that correlate with loss of the tumor suppressor phenotype in these cells may be illustrative of a general mechanism relating cell shape, differentiation, and cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 23003-02 LMC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell Senescence, Carcinogenesis, and Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: J.C. Barrett, Research Chemist, LMC, NIEHS

Others: A. Futreal, predoctoral IRTA Fellow, LMC, NIEHS
C. Afshari, predoctoral IRTA Fellow, LMC, NIEHS
A. Annab, Biologist, LMC, NIEHS
B. Burkhart, Biologist, LMC, NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Cellular Carcinogenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

3.5

PROFESSIONAL:

1.5

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Cancer remains one of the major health problems associated with aging, yet the role of the aging process in cancer remains to be determined. One approach to this problem is to study aging at the molecular level using cellular models of aging, which may provide insights into both the cancer and the aging processes. We have shown that defects in the senescence program in cancer cells can be corrected by introduction of human chromosome 1 from normal cells into immortal cells. Cellular senescence is a state of irreversible cell cycle arrest in which normal cells fail to enter into DNA synthesis upon serum stimulation. We examined whether proteins required for G₁/S cell cycle progression were irreversibly down-regulated in senescent human fibroblasts. In contrast to young cells where both forms of MAP-kinase were phosphorylated on tyrosine in response to serum, the p42^{MAP-kinase} was not tyrosine phosphorylated upon serum stimulation, whereas p44^{MAP-kinase} was phosphorylated on tyrosine in serum-starved or serum-stimulated senescent cells. Cdc2 and cyclin A mRNAs were completely down-regulated in senescent fibroblasts. Clones expressing the transfected human cyclin A or cdc2 genes senesced at a population doubling similar to controls. However, significant extension of life span were seen in cells that expressed both the transfected human cyclin A and cdc2 genes, suggesting that these two proteins may be important in controlling the life span of cells. We investigated the possible role of phosphatases in senescence. Treatment of quiescent hamster and human fibroblasts with phosphatase inhibitors (sodium orthovanadate or okadaic acid) allowed cells to progress from G₀/G₁ arrest to S-phase. In phosphatase inhibitor-treated quiescent Syrian hamster embryo fibroblasts, phosphorylation of RB and MAP-kinase proteins and induction of cdc2 protein accompanied this progression to DNA synthesis, similar to the effects of serum or mitogen treatment. Phosphatase inhibitors could also override the block to DNA synthesis in senescent cells. This suggests that protein phosphatases may play a role in the negative regulation of cell growth and maintenance of growth arrest.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 23004-01 LMC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.)

Investigation of the Immunomodulatory Activity of Swainsonine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: K. Olden, Director, NIEHS

Others: P. Breton, Visiting Fellow, LMC, NIEHS

P. Das, Visiting Associate, LMC, NIEHS

M. George, Biologist, LMC, NIEHS

COOPERATING UNITS (if any)

Howard University Cancer Center, Washington, D.C.

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Regulation of Hematopoiesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The indolizidine alkaloid, swainsonine, has previously attracted interest for its potential as an anticancer agent with reports that it 1) inhibits tumor growth and metastasis, 2) augments natural killer- and macrophage-mediated tumor cell killing, and 3) stimulates bone marrow cell proliferation. The objective of this study is to determine the potential clinical utility of this compound as a bone marrow proliferative agent. An in vitro clonal assay for murine hematopoietic progenitor cells was used to examine the effect of swainsonine on the toxicity of chemotherapeutic agents. Preliminary results with nontumor bearing mice indicate that swainsonine can be used to prevent the toxic effect on bone marrow of drugs such as methotrexate and 5-fluorouracil. We are preparing to test the effects of swainsonine on bone marrow cell proliferation and immune system functions in tumor bearing animals. Additional in vivo studies have been performed to ascertain the optimal dosage and time course of administration of swainsonine for maximum protective effect. Further studies are planned to characterize the effect of swainsonine on other chemotherapeutic drugs and to optimize the regimen of administration of swainsonine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25001-15 LMC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Mutagenesis in Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: J.C. Barrett, Research Chemist, LMC, NIEHS

Others: P. Lamb, Biologist, LMC, NIEHS

COOPERATING UNITS (if any)

Nippon Dental University, Tokyo (Dr. T. Tsutsui)
Laboratory of Reproductive and Development Toxicology, DIR (Dr. J. McLachlan)
Mt. Sinai Hospital (Dr. N. Suzuki)

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Cellular Carcinogenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.25

PROFESSIONAL:

0.25

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Most chemical carcinogens induce DNA damage and are mutagenic at specific genetic loci; however, certain carcinogens (including the human carcinogens diethylstilbestrol (DES), asbestos, arsenicals and benzene) usually do not induce gene mutations. We have examined the ability of these chemicals to induce morphological transformation, gene mutations and chromosome mutations in Syrian hamster embryo (SHE) cells in culture. We have previously proposed that the mechanism of action of DES is related to its ability to induce numerical chromosome changes, i.e., aneuploidy. Currently, DES-induced aneuploidy is being examined in the newborn mouse genital tract to test whether these changes occur in vivo in the target tissue. The mechanism of another important human carcinogen, asbestos, was also examined. We have proposed that asbestos induces cell transformation due to its ability to induce chromosomal changes. We have identified a possibly novel transforming oncogene in human mesotheliomas, and currently we are cloning this gene. Sodium arsenite and sodium arsenate are inactive as gene mutagens but are potent inducers of cell transformation but is a weak gene mutagen. This chemical is a very effective inducer of aneuploidy in this system. These results further support our hypothesis that cell transformation involves a chromosomal mutation and suggest an important role for carcinogen-induced aneuploidy in carcinogenesis. Di(2-ethylhexyl)phthalate (DEHP), a commonly used plasticizer, induces peroxisome proliferation in liver cells and hepatocellular carcinomas in rodents. We have shown that DEHP induces morphological transformation, chromosome aberrations, and peroxisome proliferations of cultured Syrian hamster embryo (SHE) cells. The transformation frequency and chromosomal aberrations by DEHP was enhanced in the presence of rat liver post-mitochondrial supernatant. The results suggest a possible involvement of genetic damage by DEHP metabolites in the induction of transformation of SHE cells. No clear relationship between induction of peroxisome proliferation and cell transformation was observed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25031-06 LMC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Tumor Suppressor Genes and Oncogenes in Chemical Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: J.C. Barrett, Research Chemist, LMC, NIEHS

Others: J.-T. Dong, Visiting Fellow, LMC, NIEHS
S. Kodama, Visiting Associate, LMC, NIEHS
H. Bivins, Biologist, LMC, NIEHS

COOPERATING UNITS (if any)

Tottori University (Dr. M. Oshimura)

LAB/BRANCH

Laboratory of Molecular Carcinogenesis

SECTION

Cellular Carcinogenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There is increasing evidence for the existence of a family of tumor suppressor genes that are involved in different cancers. Using a technique known as microcell-mediated chromosome transfer, the Laboratory of Molecular Carcinogenesis, in collaboration with Dr. Mitsuo Oshimura (Tottori University, Japan), has mapped tumor suppressor genes involved in human and rodent cancers to chromosome 1 (endometrial cancer, fibrosarcomas), chromosome 3 (renal cancer, lung cancer), chromosome 6 (endometrial cancer), chromosome 7 (choriocarcinoma), chromosome 9 (endometrial cancer), and chromosome 11 (cervical cancer, Wilms' tumor, rhabdomyosarcoma, lung cancer, fibrosarcoma). To map tumor suppressor genes for lung adenocarcinomas, we have introduced various normal human chromosomes into the A549 tumor cell line by microcell-mediated chromosome transfer to test which chromosomes had the ability to suppress tumorigenicity. These results indicate that chromosome 3 and 11 can suppress the tumorigenicity of A549 lung adenocarcinoma cells. Previous studies using somatic cell hybridization of highly metastatic and nonmetastatic rat prostatic cancer cells demonstrated that the resultant hybrids were nonmetastatic if all of the parental chromosomes were retained. Somatic hybrid segregants which underwent nonrandom chromosomal losses reexpressed high metastatic ability. These results demonstrated that there are gene(s) whose expression can suppress metastatic ability of prostatic cancer cells. To identify the location of homologous gene(s) in the human, specific human chromosomes were introduced into highly metastatic rat prostatic cancer cells using the microcell-mediated chromosome transfer. Introduction of human chromosome 11 into highly metastatic rat prostate cancer cells results in suppression of metastatic ability without suppression of the in vivo growth rate or tumorigenicity of the hybrid cells. Spontaneous deletion of portions of human chromosome 11 in some of the clones delineated the minimal portion of human chromosome 11 capable of suppressing prostatic cancer metastases as the region between 11p11.2-13 but not including the Wilms' tumor-1 locus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21016-11 LMG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Enzymes Involved in DNA Repair and Meiosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: M. A. Resnick Supv. Research Geneticist LMG NIEHS

Other: K. Lewis IRTA Fellow LMG NIEHS
E. Perkins NIH Fellow LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues

☐ (a1) Minors

☒ (c) Neither

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

From studies of DNA repair and recombination, it is apparent that nucleases play a major role in both of these processes. In the yeast *S. cerevisiae* the RAD52 gene is essential for the repair of DNA double-strand breaks (DSB), mitotic recombination and for the successful completion of meiosis. Although the function of RAD52 remains unclear, RAD52 may play a role in controlling the levels of a 72 kD nuclease (RhoNUC). From Western blot analysis, the levels of RhoNUC are greatly decreased in rad52 mutants suggesting that this nuclease is under the control of RAD52. The gene encoding RhoNUC (RNC1) has been cloned and sequenced. OFAGE analysis placed the gene encoding RhoNUC on chromosome XI. Examination of the deduced primary sequence reveals that RhoNUC is a chimeric protein with the N-terminal region exhibiting extensive homology with the ras/rho superfamily of GTP binding proteins while the C-terminus appears to encode the nuclease function. Hence, this protein has been named RhoNUC for rho-associated nuclease. Analysis of RNC1::lacZ gene fusions reveals that the control of RhoNUC by RAD52 does not occur at the level of transcription suggesting that the control is at the post-translational level. Although overexpression of RNC1 yields no significant genetic phenotype, the overexpression does cause the cells to enlarge and their nuclei appear enlarged and diffuse. Interestingly, strains carrying deletions for both RAD52 and RNC1 have nearly wild-type levels of direct repeat recombination when measured either on plasmids or on the chromosome (strains carrying only a deletion of RAD52 exhibit recombination levels approximately 100-fold lower than wild-type). From these observations, we believe that RhoNUC represents a newly identified class of proteins exhibiting both a cellular signalling function (G-protein activity) and a DNA metabolic activity (nuclease activity involved in recombination).

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

701 ES 21091-07 IMG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Effects of DNA Lesions on Untargeted DNA Metabolic Events

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. A. Resnick Supv. Research Geneticist LMG NIEHS

Others: C. Bennett Visiting Associate LMG NIEHS
M. Davenport Biologist LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.8

PROFESSIONAL:

1.8

OTHER:

0

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects ☐ (b) Human tissues

☐ (a1) Minors

☒ (c) Neither

☐ (a2) Interviews

SUMMARY OF WORK (Use standard/unreduced type. Do not exceed the space provided.)

Following DNA damage, cells of the yeast Saccharomyces cerevisiae undergo a RAD9-dependent arrest in the G2 phase of the cell cycle. We are attempting to elucidate the molecular mechanism that allows DNA lesions to signal global cellular responses to DNA damage. The effects of an enzymatically induced double-strand break at a 45 bp YZ sequence (from MATYZ) in a nonyeast region of a dispensible single copy plasmid were examined. Induction of a persistent unrepaired break in trans results in nearly complete loss of plating efficiency even though the plasmid is dispensible. This loss of plating efficiency is partially dependent on the RAD9 gene product. Examination of single cells from rapidly cycling unsynchronized populations of RAD⁺ and rad9 cells following induction of the DSB indicated that over one-third of the cells (including both unbudded [G-1] and budded [S + G-2] cells) were inhibited from further cell division. The remaining cells of a RAD⁺ strain give rise primarily to microcolonies containing permanently arrested aberrantly shaped cells. Microcolonies were not observed in the rad9 background which accounts for the increased plating efficiency of this strain. This system provides a means for studying the signalling effects of a DNA lesion as well as designing strategies for modulating cell proliferation. In a study using the above methodology, we have investigated whether a site specific DSB can be used to physically map specific genes within the yeast genome. We have constructed and integrated LEU2YZ, URA3YZ and RNC1YZLEU2 targeting vectors into the yeast genome. Following integration, the yeast were transformed with a selectable plasmid containing the HO endonuclease gene under gal control. Galactose induced a size specific chromosomal fragmentation at the LEU2YZ and URA3YZ integration sites on chromosomes III and V respectively corresponding to the known physical location of these genes. These results indicate that this approach can be used successfully to physically map cloned human or yeast genes within the yeast genome or upon YACs containing human DNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21122-04 LMG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genomic Stability and Recombinational Interactions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. A. Resnick	Supv. Research Geneticist	LMG NIEHS
	G. Porter	IRTA	LMG NIEHS
	S. D. Priebe	NRC Fellow	LMG NIEHS

COOPERATING UNITS (if any)

T. Nillsson-Tillgren, University of Copenhagen, Denmark
M. Radman, Univ. of Paris, Paris, France

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.2

PROFESSIONAL:

2.2

OTHER:

0

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors ☒ (c) Neither
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrevoked type. Do not exceed the space provided.)

Recombination is required for the repair of many types of lesions and it can be a source of genetic diversity. DNA sequence divergence (homoeology) is expected to impede recombination efficiency because of constraints inherent in the biochemical reactions. Ancillary genetic factors such as DNA-mismatch repair are also expected to affect recombination between homologous DNAs. We are examining the requirements and consequences of recombination between divergent DNAs to gain insight on mechanisms of recombination, mechanisms of chromosome rearrangements, and possibly mechanisms of initiation of carcinogenesis. We have previously demonstrated that recombinational repair in plasmid DNA occurs less efficiently if the chromosomal DNA available as the template for repair is homologous rather than nonhomologous. Proficiency for DNA mismatch repair appears to have little or no effect on the frequency or products (examined at the molecular level) of these recombination events. Based on these results, we suggest updated versions of models for recombinational repair. We are extending this analysis to defects in other DNA repair functions. To further elaborate the effect of mismatch repair on recombination between diverged DNAs, we have studied model heteroduplex plasmids which resemble structures proposed as intermediates in recombination between diverged DNAs. We previously demonstrated that a mixture of closely related heteroduplex plasmids survive transformation at nearly the same rate in methyl-directed mismatch repair (MMR)-proficient or deficient *E. coli*. We are developing methods of producing subfractions of model heteroduplex plasmids to study the effects of specific configurations on susceptibility to attack by MMR; the exact configuration is an important parameter in models of recombination repair. These experiments are currently being extended to *S. cerevisiae*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61037-08 LMG

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mechanism of DNA Replication in Eucaryotes: Yeast as a Model System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Sugino	Visiting Scientist	LMG NIEHS
Others:	A. Morrison	Visiting Scientist	LMG NIEHS
	K. Kitada	Visiting Associate	LMG NIEHS
	J. Sebastian	Senior Staff Fellow	LMG NIEHS
	P. Ropp	IRTA Fellow	LMG NIEHS
	A. B. Clark	Biologist	LMG NIEHS

COOPERATING UNITS (if any) Dr. L. H. Johnston, Group Leader, Lab. Yeast Genetics, Nat. Inst. for Med. Res., London, England; Prof. B.-K. Tye, Section of Biochemistry, Molec. and Cell Biol., Cornell Univ., Ithaca, NY; Prof. R. A. Bambara, Dept. of Biochem., Univ. of Rochester, Rochester, NY

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Mutagenesis Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS: 5.0	PROFESSIONAL: 4.0	OTHER: 1.0
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CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors ☒ (c) Neither
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

Various temperature-sensitive DNA polymerase II mutants of *S. cerevisiae* have been characterized. The purified DNA polymerase II from the mutants exhibited temperature-sensitive DNA polymerase activity, while in vivo chromosomal DNA synthesis was greatly reduced at the restrictive temperatures. Furthermore, these mutant cells had a single-base-pair change which generates a single amino acid change in the DNA polymerase II gene. These results proved that *S. cerevisiae* DNA polymerase II participates in its chromosomal DNA replication. By using the PCR technique, we have cloned the DNA polymerase gene which has a high homology to *S. cerevisiae* DNA polymerase II from a fission yeast *S. pombe* and from *Drosophila melanogaster* and their partial nucleotide sequences have been determined.

The CDC7 gene product is required for the last step before initiation of chromosomal DNA replication in *S. cerevisiae*. In the previous year, we have shown genetically that the CDC7 gene product (a protein kinase) directly interacts with the DBF4 gene product which is also required for initiation of chromosomal DNA replication and suggested that the DBF4 protein activates the protein(s) which is needed for initiation of chromosomal DNA replication. In this year, we have obtained biochemical evidences for the direct interaction between the DBF4 protein and the CDC7 protein. Furthermore, an active form of the CDC7 protein kinase consisted of the CDC7 and DBF4 polypeptides. These data further substantiated our proposal that the DBF4 protein works as a CDC28 (CDC2) cyclin and modulates activity of the CDC7 protein kinase. Finally, we have isolated, sequenced, and characterized the CDC5 gene which interacts with DBF4, CDC15, and DBF2 gene products.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61039-08 LMG

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)
Mechanisms of DNA Recombination and Repair in the Yeast *Saccharomyces cerevisiae*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
PI: A. Sugino Visiting Scientist LMG NIEHS

Others: A. Morrison Visiting Scientist LMG NIEHS
K. Shimizu Visiting Fellow LMG NIEHS

COOPERATING UNITS (if any)
Prof. H. Ikeda, Dept. of Molecular Genetics, Inst. of Med. Science, The Univ. of Tokyo, Minato-Ku, Tokyo, Japan; Dr. Eric Ackerman, Lab. of Biochemistry, NIDDKD, NIH, Bethesda, MD 20892

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Laboratory of Molecular Genetics

SECTION
Mutagenesis Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS: 1.25	PROFESSIONAL: 1.25	OTHER: 0
----------------------------	-----------------------	-------------

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors ☒ (c) Neither
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unabbreviated types. Do not exceed the space provided.)

It has been believed that DNA polymerase β is required for DNA repair reaction in mammalian cells. Although other DNA polymerases are well conserved functionally and structurally between yeast and mammalian cells, so far no DNA polymerase β -like DNA polymerase activity has not been identified in yeast cells. Thus, we have tried to identify a new DNA polymerase activity that resemble to mammalian DNA polymerase β . We successfully identified in the crude extracts of *S. cerevisiae* cells and partially purified such activity. Although its apparent molecular weight of the DNA polymerase polypeptide was considerably larger than that of DNA polymerase β , its biochemical properties were very similar to those of DNA polymerase β . To study this new DNA polymerase's function, we have been trying to clone a gene encoding the DNA polymerase using antibody against the purified DNA polymerase and the PCR technique.

DNA helicase is believed to be required not only for DNA replication, but also for DNA repair and recombination. We have identified and purified a new DNA helicase activity. The apparent molecular weight and biochemical properties of the purified polypeptide, and genetical studies indicated that this DNA helicase is distinguished from the previously identified DNA helicases in yeast, suggesting that it is encoded by a new gene.

Using the cloned *S. cerevisiae* *DST2* which encodes DNA strand transfer protein β as a hybridization probe, we have identified two homologs of *DST2* in *S. pombe*. One of them which encodes a 120-kDa polypeptide was found to be an essential gene for *S. pombe* cell growth, suggesting that it plays an important role in *S. pombe*. Using the 120-kDa polypeptide gene as a probe, we have also identified and cloned a *S. cerevisiae* homolog which encodes a 120-kDa polypeptide. Like *S. pombe*, the *S. cerevisiae* 120-kDa polypeptide gene is essential for cell growth. To find the function of the 120-kDa polypeptide in the cell, we have isolated various temperature-sensitive mutants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65034-08 LMG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

The Specificity of Spontaneous and Induced Mutation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. M. Schaaper Visiting Scientist LMG NIEHS

Others: R. L. Dunn Biologist LMG NIEHS
A. Oller IRTA Fellow LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.50

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

In this project the mechanisms of mutagenesis are investigated through the detailed study of its specificity. This is pursued by DNA sequencing of mutations in the *Escherichia coli* *lacI* gene, occurring either spontaneously or after treatment with a mutagen. The specificity of mutation provides a way to dissect the components of mutagenesis since different pathways and mechanisms produce characteristic patterns of mutation with regard to type and DNA sequence dependence. The *lacI* gene encodes the repressor of the *lac* operon and forward mutations to *lacI*⁻ can be scored based on their constitutive expression of the operon. The mutant genes are transferred by *in vivo* recombination to a single-stranded phage vector and sequenced. In order to understand the pathways that contribute to spontaneous mutations, we have analyzed spectra of mutations in the *E. coli* wild-type strain and in its *mutH*, *mutL*, *mutS*, *mutT*, *mutD* and *mutY* derivatives. This has allowed us to determine not only the nature of spontaneous mutations, but also the nature of primary DNA replication errors and the specific contributions of exonucleolytic proofreading and DNA mismatch repair. For induced mutagenesis, we have focused on mutagenesis by ultraviolet light (UV). The nature and location of the UV-induced mutations should provide insights into the responsible mechanisms. We, and others, have shown that the large majority of UV-induced mutations occurs at adjacent pyrimidines in the DNA sequence, implicating cyclobutane pyrimidine dimers and (6-4) pyrimidine-pyrimidone lesions (the two predominant UV-induced lesions in DNA) as premutagenic lesions. Such analysis has been performed in excision-repair proficient and deficient strains as well as *mfd* strains, deficient in the coupling between DNA transcription and DNA repair. These studies allow a further understanding of the pathways by which UV-induced lesions are processed through transcription, repair, and replication, ultimately leading to a mutation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65046-06 LMG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Accuracy of DNA Replication in vitro

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. A. Kunkel Research Geneticist LMG NIEHS

Others: J. D. Roberts Senior Staff Fellow LMG NIEHS
D. C. Thomas Senior Staff Fellow LMG NIEHS
J. D. Bell Senior Staff Fellow LMG NIEHS
A. Sugino Visiting Scientist LMG NIEHS
D. C. Nguyen Biologist LMG NIEHS
S. Izuta Visiting Associate LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

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Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

3.8

PROFESSIONAL:

2.9

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrevoked type. Do not exceed the space provided.)

Replication of the diploid human genome involves a series of complex reactions occurring in at least four phases: initiation of synthesis at origins, elongation on the leading strand, elongation on the lagging strand and replacement of RNA primers with DNA. Current models suggest that more than one DNA polymerase is required for replication, that the proteins that start or finish chains may be different than those that perform the bulk of chain elongation and that the proteins that replicate the leading and lagging strands may be different. Our long-term goal is to define the fine structure of DNA replication fidelity. To do so, we are examining the fidelity of DNA synthesis catalyzed by eukaryotic DNA polymerases α , β , δ , and γ and the fidelity of bidirectional DNA replication by the multiprotein replication apparatus in extracts of human HeLa cells. We have found that polymerases have distinctly different error rates and specificities, which have implications for their roles in the various stages of DNA replication. We have described base substitution and frameshift error rates for the replication apparatus and obtained data suggesting that exonucleolytic proofreading occurs during replication of both the leading and lagging strands. We have found different error rates on the two strands for frameshift errors at two non-reiterated sequence positions. We have begun to examine a large number of errors and template positions using a forward mutation assay. In order to better understand the effects of known mutagens and carcinogens on the fidelity of DNA synthesis, we have also recently initiated studies of replication fidelity with DNA molecules containing defined lesions, including psoralen monoadducts, UV photoproducts and AAF adducts. We intend to continue these studies to define error rates for each of the four phases of replication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 ES 65047-06 LMG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)
Fidelity of Retroviral Reverse Transcriptases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. A. Kunkel Research Geneticist LMG NIEHS

Others: K. Bebenek Staff Fellow LMG NIEHS
K. Eckert IRTA Fellow LMG NIEHS
D. C. Thomas Senior Staff Fellow LMG NIEHS
J. C. Boyer Staff Fellow LMG NIEHS

COOPERATING UNITS (if any)

Samuel Wilson, Research Biochemist, LB, NCI
Brendan Larder, Wellcome Research Laboratories, Kent, England

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Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.6

PROFESSIONAL

2.6

OTHER:

0

CHECK APPROPRIATE BOXES:

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Sequence variation in the genome of the human immunodeficiency virus (HIV-1) results from inaccurate replication by reverse transcriptase. This enzyme is error-prone during synthesis in vitro with DNA templates. However, the fidelity of RNA-dependent DNA synthesis relevant to minus-strand replication in the virus life cycle had not been examined previously. This year we developed an assay system to determine the fidelity of transcription and reverse transcription and used it to compare the fidelity of DNA synthesis by the HIV-1 reverse transcriptase with RNA and DNA templates of the same sequence. Overall, fidelity was several-fold higher with RNA than with DNA. Sequence analysis of mutants generated with the two substrates revealed that differences in error rates were substantial for specific errors. Fidelity with RNA was more than 10-fold higher for substitution and minus-one nucleotide errors at five different homopolymeric positions. Since such errors likely result from template-primer slippage, this result suggests that misaligned intermediates are formed and/or utilized less frequently with an RNA template-DNA primer than with a DNA template-DNA primer. The results also suggest that HIV-1 RT synthesis with an RNA template-DNA primer was error-prone during incorporation of the first two nucleotides, perhaps due to aberrant enzyme-substrate interactions as synthesis initiates. The unequal error rates with RNA and DNA templates suggest that mistakes during minus- and plus-strand DNA synthesis may not contribute equally to the mutation rate of HIV-1. The data also provide estimates of substitution and frameshift error rates during transcription by T7 RNA polymerase. We also completed studies of the effects of AZT metabolites on normal cellular replication and on mismatch repair and studies of the RT from AZT-resistant virus clones. We will continue to focus on elucidating the mechanisms responsible for the error-proness of HIV-1 RT, in the hope that this will provide insights into the interaction of the enzyme's active site with its substrates that may be useful in designing RT targeted drugs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65055-04 LMG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Bacteriophage T4 Antimutator Mutations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. W. Drake

Chief, LMG

LMG NIEHS

Others:

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.1

PROFESSIONAL

0.1

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues

☐ (a1) Minors

☒ (c) Neither

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unnumbered type. Do not exceed the space provided.)

Work was continued during the fiscal year to complete final writing of papers.

Mutations in the bacteriophage T4 DNA polymerase gene sometimes reduce spontaneous mutation rates, in which case they have been called antimutator mutations. It has gradually become clear that while some kinds of mutations are reduced in frequency in such strains, other kinds of mutations are increased. Both structural and evolutionary considerations suggested that a decrease in the total mutation rate is unlikely to be achievable by a single missense mutation except perhaps at the cost of a reduced rate of DNA synthesis. The average mutation rate over a target of roughly 5 kilobase pairs was determined in a set of 12 antimutator mutations. In each case, the rate was indistinguishable from or greater than the wild-type mutation rate. This project is now completed and terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZD1 ES 65070-02 LMG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (20 characters or less. This must fit on one line between the borders.)

Probing Structure-Function Relationships with DNA Polymerases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator; Name, title, laboratory, and institute affiliation)

PI: I. A. Kunkel Research Geneticist LMG NIEHS

Others: J. Bell Senior Staff Fellow LMG NIEHS
K. Eckert Staff Fellow LMG NIEHS
D. Nguyen Biologist LMG NIEHS

COOPERATING UNITS (if any)

Catherine M. Joyce, Yale University Medical School, New Haven, CT
Samuel H. Wilson, LB, NCI, NIH, Bethesda, MD

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Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.9

OTHER:

0.1

CHECK APPROPRIATE BOXES:

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unstructured type. Do not exceed the space provided.)

We are using mutant enzymes obtained by recombinant DNA technology to examine the relationship between the structural and kinetic properties of DNA polymerases and their fidelity. The fidelity of exonuclease-deficient Klenow polymerase has been examined as a function of reaction pH. Overall fidelity increase more than 10-fold as the pH is lowered from 7.6 to 6.2, but rates are not affected equally for all errors. These non-random effects are consistent with steady-state kinetic analyses indicating that lowering the pH lowers the efficiency of mispair extension synthesis. Variations in the termination probabilities of more than 20-fold were observed at individual sites along the template DNA as a function of pH, and increased termination correlated with increased fidelity. These data suggest that high fidelity at low pH may result from both decreased formation of premutational intermediates due to the increased processivity of the polymerase and from decreased fixation of mutations due to decreased extension of misaligned or mispaired intermediates. We also extended the analysis to two new mutants in which a tyrosine residue at position 766, in the "O helix" of the polymerase active site, was changed to an alanine or serine. Both mutants have lower fidelity, suggesting that the tyrosine residue, known to bind dNTP substrates, is important for determining base selectivity. We are in the process of defining the detailed error specificity of these altered polymerases. We are also examining mutant derivatives of several other recombinant DNA polymerases, including HIV-1 reverse transcriptase, DNA polymerase β , T7 DNA polymerase and T4 DNA polymerase. Analyses of these enzymes, for which considerable structural and/or kinetic data are available, should improve our understanding of accurate DNA synthesis and how fidelity is affected by DNA adducts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65071-02 LMG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Reviews of Mutation and DNA Repair

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. W. Drake Chief, LMG LMG NIEHS

Others:

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.60

PROFESSIONAL:

0.60

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Extensive critical reviews have been prepared in three areas that have not been recently examined. The first considers selected aspects of spontaneous mutation, with emphasis on base mispairing schemes, directed mutation, the processing of repeated sequences in higher eukaryotic genomes, and the evolution of mutation rates. The second covers the entire literature on mutation research employing T-even bacteriophage model systems. The third covers the entire literature on DNA repair employing T-even bacteriophage model systems. This project is completed and the reviews are in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65072-02 LMG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Human Genome Project: Artificial Chromosome Stability and Mapping in Yeast

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. A. Resnick	Supv. Research Geneticist	LMG NIEHS
Others:	J. Westmoreland	Biologist	LMG NIEHS
	N. Kouprina	Visiting Scientist	LMG NIEHS
	V. Laronov	Visiting Associate	LMG NIEHS
	M. Eldarov	Visiting Associate	LMG NIEHS
	C. Bennett	Visiting Associate	LMG NIEHS
	E. Perkins	NIH Postdoctoral Fellow	LMG NIEHS

COOPERATING UNITS (if any)

J. Michel-Vos, University of North Carolina

LAB/BRANCH

Laboratory of Molecular Genetics

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

4.1

PROFESSIONAL:

4.1

OTHER:

0

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unbolded type. Do not exceed the space provided.)

The mapping and sequencing of the human genome is a world-wide effort with many anticipated health benefits. A cornerstone of the Human Genome Project (HGP) is the use of yeast artificial chromosome (YAC) vectors for the cloning of large chromosome fragments. It is essential for the HGP that human DNA within YACs be stable within yeast. A major source of artefacts as we have shown arises from the repetitive nature of the DNA. These are potential substrates for co-cloning events and transformation-associated and mitotic recombination since recombination between diverged DNAs has been demonstrated in yeast. Some YACs exhibit considerable internal recombinational repair. We have suggested that the extent of repeats, even if diverged, may be indicated by the sensitivity of YACs to ionizing radiation induced loss and that the survival of YACs is dictated by the amount of recombinational repair between the diverged DNAs (currently under study). Small repeats that surround large inverted repeats (such as Alu's) may be sites of excision, as we have shown for Tn5 DNA in yeast.

Problems in replication may also contribute significantly to YAC instability. Two potential sources of replication problems are, 1) insufficient functional origins of replication in the long tracts of mammalian DNA, and 2) possible deletions of sequences flanked by inverted repeats, which have been shown to facilitate replication bypass. We have shown that specific replication mutants can enhance recombination between large repeats in chromosomes and can stimulate up to 100-fold excision involving small repeats in Tn5.

The goals of the project are, 1) improvement of cloning methods and recipient strains, 2) improvement of YAC stability utilizing replication and recombination mutants and gene products, and 3) development of in vivo fragmentation systems for the precise physical mapping of sequences of interest within YACs. We are also merging YAC cloning systems with mammalian viral cloning systems for the easy transfer of large fragments to human cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES : PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65073-02 LMG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Molecular Mechanisms of DNA Repair and Recombination in Yeast

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: M. A. Resnick Supv. Research Geneticist LMG NIEHS

Other: E. Perkins NIH Fellow LMG NIEHS
J. Westmoreland Biologist LMG NIEHS

COOPERATING UNITS (if any)

D. Gordenin, Dept. of Genetics, Leningrad University, USSR

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.8

PROFESSIONAL:

0.6

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unswelled type. Do not exceed the space provided.)

We are investigating the unique chromosomal metabolic events associated with meiosis and the repair of chromosomes following exposure to DNA damaging agents. Many of the genes necessary for the repair of DNA double strand breaks (DSB) are required for the successful completion of the meiotic cycle. In particular the RAD52 gene is essential for the repair of DSBs and the successful completion of meiosis. Furthermore, RAD52 appears to influence mitotic recombination. To investigate the role of a RAD52, we have created strains containing a chromosomal resident deletion of RAD52 and a RAD52 gene under the control of the yeast GAL1 promoter. This allows us to tightly regulate the expression of the RAD52 gene. We have found that for the repair of DSBs the presence of RAD52 protein is required prior to treatment with ionizing radiation. Interestingly, the presence of a previously isolated nuclease (RhoNUC) appears to be influenced by RAD52, however, this control appears to be post-translational. In order to identify other proteins which may interact with RAD52 we have begun screening a "protein interaction" library.

The possible role of RAD52 during replication is also being investigated. Using a unique system whereby we measure the frequency of "excision" of a bacterial transposon Tn5 placed into the yeast LYS2 gene, we have found that mutations in the gene encoding DNA polymerase I or III lead to high levels of Tn5 "excision". No increase is observed in DNA polymerase II mutants. The elevated levels of "excision" seen in polIII or polI strains is reduced when a null mutant of RAD52 is introduced into these strains. This suggests an interaction between the enzymes proposed to be responsible for DNA lagging strand synthesis and RAD52.

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65074-01 LMG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antimutator Mechanisms in *Escherichia coli*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. M. Schaaper

Visiting Scientist

LMG NIEHS

Others: R. L. Dunn

Biologist

LMG NIEHS

I. Fijalkowska

Visiting Fellow

LMG NIEHS

J-Y. Mo

Visiting Fellow

LMG NIEHS

COOPERATING UNITS (if any)

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Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.5

OTHER:

0.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues

☐ (a1) Minors

☒ (c) Neither

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Antimutator strains (i.e., strains that have lower mutation rates than the parental wild-type strain) are important tools in elucidating the precise mechanisms by which organisms produce mutations. In general, antimutators can be thought of as organisms that possess increased efficiency in certain cellular mutation-prevention systems. The well-defined genetics and molecular biology of the bacterium *E. coli* permit a systematic search for mutants that are thus affected. For example, by means of specific tests, mutants may be isolated that produce less errors of replication (by virtue of increased efficiency of DNA polymerase base selectivity, increased exonucleolytic proofreading, or increased DNA mismatch repair). Subsequent demonstration that these DNA replication mutants are antimutators for overall spontaneous mutagenesis would provide strong evidence that errors of DNA replication are a main source of spontaneous mutation. Analogously, mutants might be isolated possessing increased resistance against DNA damage from cellular metabolites, such as oxygen radicals. A demonstration that such mutants possess reduced overall mutation rates may be used to argue that oxidative damage is a main contributor to spontaneous mutation. The usefulness of such pathway-specific antimutators is not limited to spontaneous mutation but extends to the many forms of induced mutation. Using this approach, we have developed a specific assay to isolate mutants of *E. coli* that display reduced rates of DNA replication errors. These strains were found to carry a mutation in the *dnaE* gene, which encodes the DNA polymerase III that is primarily responsible for the (faithful) replication of the bacterial chromosome. It is our expectation that these strains will allow us to (i) define the specific contribution of DNA replication errors to spontaneous mutation, (ii) further define the mechanisms by which DNA polymerases can replicate DNA with high fidelity.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 ES 70090-09 LMIN

PERIOD COVERED

October 1, 1991 to September 30, 1992

(Title must fit on one line between the borders)

Neuroendocrine and Neurochemical Regulation of Gonadal Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Andres Negro-Vilar	Chief	LMIN	NIEHS
Others:	I. Merchenthaler	Visiting Scientist	LMIN	NIEHS
	W. C. Wetsel	Senior Staff Fellow	LMIN	NIEHS
	F. Lopez	Visiting Scientist	LMIN	NIEHS
	M. Moretto	Visiting Associate	LMIN	NIEHS
	M. Wisniewski	Biologist	LMIN	NIEHS
	E. Meade, Jr.	Biologist	LMIN	NIEHS
	L. E. Fogel	Biological Aid	LMIN	NIEHS

COOPERATING UNITS *(if any)*

The Salk Institute, La Jolla, California

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Neuroendocrinology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.8

PROFESSIONAL:

1.5

OTHER

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

A fundamental problem in neuroendocrinology consists of the locus and/or entity responsible for ultradian rhythms related to the genesis of pulsatile secretory patterns. Recently, we have shown that an immortalized LHRH neuronal cell line (GT1 cells) has the ability to release LHRH in a pulsatile manner, thereby raising the possibility that the LHRH pulse generator may reside within LHRH neurons. The pulsatile secretory activity of these neurons suggests a degree of synchronization of activity to allow for the generation of episodic secretion. Under perfusion conditions, GT1-7 neurons display a syncytial-like architecture, establishing GAP junctions and synaptic-like contacts. It is still unclear, however, how secretory episodes emerge over an otherwise noisy background of firing cells connected without any apparent predetermined organization. Recent studies have identified nitric oxide(NO) as the endothelium-derived relaxing factor. NO has been localized to neurons and described as an orthodox neurotransmitter based on its particular physico-chemical properties such as an extremely fast diffusibility. We have tested the hypothesis of whether NO is a putative candidate to synchronize LHRH neurons using functional and morphological approaches. Two different strategies were utilized to evaluate the possible participation of NO in the genesis of pulsatile LHRH secretion. Oxyhemoglobin (Hb), a sequester of NO, and NG-monomethyl-L-arginine (L-NMMA), a competitive inhibitor of the NO synthase, were utilized to reduce NO availability or production. GT1-7 cells perfused with medium containing either Hb or L-NMMA showed a dramatic reduction or, in some cases, a complete abolition of LHRH pulsatile activity. Immunocytochemical studies have indicated that these cells stain for citrulline, a collateral product in the biosynthesis of NO by the nitric oxide synthase. These observations support the notion of an effective synthesis of NO in these neurons. In summary, these data indicate that the LHRH neuronal network may utilize NO, at least in part, as a synchronizing mechanism for exhibiting pulsatile LHRH secretion in the absence of any non-LHRH neuronal input.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 ES 70092-09 LMIN

PERIOD COVERED

October 1, 1991 to September 30, 1992

(Title must fit on one line between the borders)

Cellular and Molecular Mechanisms Mediating Peptide Hormone Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Negro-Vilar	Research Physiologist	LMIN	NIEHS
Others:	Francisco J. Lopez	Visiting Scientist	LMIN	NIEHS
	M. Moretto	Visiting Associate	LMIN	NIEHS
	E. Meade, Jr.	Biologist	LMIN	NIEHS
	L. E. Fogel	Biological Aid	LMIN	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Neuroendocrinology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.8

PROFESSIONAL:

2.2

OTHER

0.6

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

Endothelins represent a family of recently discovered peptides that are secreted from endothelial cells cultured in vitro and depict potent vasoactive properties. Of the three endothelin (ET) genes identified, ET-3 seems to be the member of the family most abundant in the brain. Since ET-3 immunoreactivity and receptors are present in the hypothalamus, the peptide could play a role in the modulation of neuroendocrine functions. In this respect, it has been recently shown that ET-3 may enhance LH secretion from pituitary cells in vitro, indicating a role for ET-3 in the regulation of reproductive functions. In these studies, we evaluate whether ET-3 participates in the central regulation of gonadal functions by controlling LHRH release. For this purpose, we analyzed the effects of the peptide on LHRH secretion using two experimental models: arcuate nucleus-median eminence (AN-ME) fragments incubated in vitro and immortalized LHRH neurons (GT1-7) incubated in a perfusion paradigm. ET-3 stimulated LHRH secretion from both AN-ME fragments incubated in a static system and GT1-7 cells in perfusion. In AN-ME incubations, ET-3-induced LHRH release appears to require PGE2 synthesis. ET-3-evoked LHRH secretion was concomitant with an increase in PGE2 release into the incubation medium; in addition, inhibition of PG synthesis with indomethacin blocked ET-3-induced secretion of both LHRH and PGE2. Furthermore, the effects of ET-3 on LHRH release were enhanced by potassium-evoked depolarization. This enhancement could not be explained by a simple additive effect of both secretagogues, indicating that the activation of other messenger systems may be required for obtaining a complete activation of the ET-3-related actions on LHRH terminals. Our data indicate that endothelins could be considered as modulators of LHRH secretion and that PGE2 is a possible intracellular mediator in these effects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 ES 70096-08 LMIN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)

Regulation of Pulsatile Gonadotropin Secretion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michael D. Culler Expert LMIN NIEHS

Others: Carl Paschall Biologist LMIN NIEHS

COOPERATING UNITS (if any)

DRTG, STB, NIEHS

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Neuroendocrinology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.0

OTHER

1.0

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

The overall goal of this on-going project is to dissect the mechanisms by which the brain and gonads interact to control gonadotropin secretion and reproductive function. During the last year we concentrated on the role of inhibin as a feedback regulator of luteinizing hormone (LH). Stimulation of ovarian function through exogenous FSH administration suppresses the preovulatory surges of both LH and FSH in a number of species including humans. This phenomenon has been demonstrated to be the result of an unknown ovarian factor that has been designated gonadotropin surge-inhibiting factor (GnSIF). We observed that inhibin immunoreactivity was found to be significantly elevated in rats receiving an FSH treatment protocol known to increase GnSIF activity. FSH-stimulation of female rats was observed to completely prevent the preovulatory LH and FSH surges; however, in FSH-stimulated rats subsequently treated with anti-inhibin serum, normal preovulatory surges were observed. These results indicate that inhibin is the factor responsible for the suppression of the preovulatory gonadotropin surges in FSH-stimulated rats and may be the long sought, clinically important GnSIF. In related studies, we demonstrated that FSH-stimulation suppressed the development of LHRH self-priming of the pituitary which occurs on proestrus following multiple LHRH exposures. Immunoneutralization of endogenous inhibin eliminated LHRH self-priming in both FSH-stimulated and normal proestrous females by increasing pituitary sensitivity to LHRH to a maximal level prior to the first exposure to exogenous LHRH. These results demonstrate the importance of inhibin as a modulator of LH secretion during the female estrous cycle and shatter the concept of inhibin as a selective regulator of FSH secretion. In other studies, we demonstrated that the newly isolated peptide, pituitary adenylate cyclase-activating peptide (PACAP), enhances the gonadotropin-releasing activity of LHRH in cultured pituitary cells. This observation suggests intriguing possibilities for PACAP both as a modulator of gonadotropin secretion and as a possible probe of intracellular cross-talk within the gonadotrophs. We have demonstrated the ability of PACAP to both stimulate cAMP production and regulate a number of secretory functions in cultured Sertoli and ovarian granulosa cells, thus further establishing PACAP as a modulator of reproductive function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90033-10 LMIN

PERIOD COVERED

October 1, 1991 to September 30, 1992 (Terminated September 30, 1991)

TITLE OF PROJECT (Title must fit on one line between the borders)

Milk Bradykinin and Kininogens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William E. Wilson	Research Chemist	LMIN	NIEHS
Others:	L. H. Lazarus	Research Chemist	LMIN	NIEHS
	A. Haugen-Strano	Bio. Lab Technician	LMIN	NIEHS

COOPERATING UNITS (if any)

K. B. Tomer and L. J. Deterding, Mass Spectrometry Branch, LMB, NIEHS

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Peptide Neurochemistry Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.5

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

Difficulties which appeared to involve co-purification and/or generation of proteolytic enzymatic activity prevented the successful purification of bradykininogen(s) from milk or nerve tissues. The lack of a suitable antiserum was also a contributing factor as the smooth muscle bioassay technique did not provide adequate specificity to confirm that the tissue kininogens were precursors of bradykinin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 90034-09 LMIN
PERIOD COVERED October 1, 1991 to September 30, 1992 (Terminated on September 30, 1991)		
TITLE OF PROJECT (Title must fit on one line between the borders) Antigenicity of PHLIP-8 and Other Physiological Peptides		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: William E. Wilson Research Chemist LMIN NIEHS Others: L. H. Lazarus Research Chemist LMIN NIEHS		
COOPERATING UNITS (if any) Animal Husbandry, NIEHS		
LAB/BRANCH Laboratory of Molecular and Integrative Neuroscience		
SECTION Peptide Neurochemistry Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK All efforts to obtain antiserum to PHLIP-8 were judged to be unsuccessful.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT			PROJECT NUMBER Z01 ES 90043-07 LMIN
PERIOD COVERED October 1, 1991 to September 30, 1992			
TITLE OF PROJECT (Title must fit on one line between the borders) Role of Zinc in Synaptic Transmission in the Hippocampal Formation			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)			
PI:	Clifford L. Mitchell	Pharmacologist	LMIN NIEHS
Others:	H. Xu	Guest Worker	LMIN NIEHS
COOPERATING UNITS (if any)			
LAB/BRANCH Laboratory of Molecular and Integrative Neuroscience			
SECTION Neurophysiology Group			
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709			
TOTAL STAFF YEARS:		PROFESSIONAL:	
0.7		0.2	
		OTHER	
		0.5	
CHECK APPROPRIATE BOXES)			
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither			
☐ (a1) Minors			
☐ (a2) Interviews			
SUMMARY OF WORK Several pieces of evidence suggest that endogenous opioids and zinc may interact to regulate neuronal excitability within the hippocampal formation. The purpose of this project is to conduct a systematic investigation into the effects of zinc on hippocampal neuronal excitability. The goal is to explain the nature of the effects of zinc and the mechanism(s) for its modulation of hippocampal excitability. First it was necessary to determine the manner in which zinc levels were to be altered. As an initial approach we chose to attempt to alter zinc levels by systemic administration of the intravital zinc chelators, dithizone and diethyldithiocarbamate (DEDTC). The biological assay used was occurrence of wet dog shakes and seizures following subcutaneous administration of kainic acid (KA). Intraperitoneal injection of dithizone (12.5-100 mg/kg) or DEDTC (100-400 mg/kg) has a profound and dose related affect on the effects of KA. When given 15 minutes after subcutaneous injection of KA, they markedly potentiate KA activity. They also produce a transient decrease in hippocampal levels of enkephalin and dynorphin. They also produce transient increases in the hippocampal levels of a number of amino acids (viz., taurine, glutamate, glutamine, and GABA). These effects are associated with reduced levels of hippocampal zinc (as measured by Timm staining of the hippocampus). We have also found that DEDTC lowers the threshold for behavioral seizures elicited by electrical stimulation of the perforant path (the major input to the hippocampal formation). However, it does not lower the threshold for electrical afterdischarge but does markedly prolong the afterdischarge. Hippocampal slices perfused with DEDTC also exhibit a lowered threshold for epileptiform bursting. This effect can be blocked by replenishing the zinc content of the tissue. These data strongly suggest that endogenous zinc plays an important role in reducing seizure activity in the hippocampus.			

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 ES 90044-07 LMIN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)

Modulation of Neuronal Function by Neuropeptides and Steroid Hormones

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Clifford L. Mitchell

Pharmacologist

LMIN

NIEHS

Others: J. S. Hong

Pharmacologist

LMIN

NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neurophysiology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.5

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK

Work in this laboratory has focused on the role of enkephalin and dynorphin in seizure activity and related sequelae. This work has implicated enkephalin as playing a major role in the elucidation of a phenomenon in rats known as "wet dog shakes" (WDS). This work has also implicated the dentate granule cells (DGCs) as being necessary for the elicitation of WDS at least with respect to induction by kainic acid or by stimulation of the perforant path (PP). We have demonstrated that stimulation of PP under conditions which elicit WDS produces a significant decrease in hippocampal levels of enkephalin and dynorphin. Moreover, intraventricular injection of either an opioid mu receptor (beta-FNA) or delta receptor (ICI174864) antagonist reduced the number of WDS elicited by PP stimulation. These data provide the first evidence that endogenous opioids are released by PP stimulation and lend further support to the notion that they play a role in regulation of hippocampal excitability. We have also demonstrated that the opioid receptor antagonist, naltrexone, when injected directly into the ventral hippocampus, produces an elevation in the threshold for eliciting wet dog shakes. We have demonstrated that destruction of dentate granule cells in the ventral, but not dorsal, hippocampal formation abolishes wet dog shaking induced by perforant path or intrahippocampal stimulation or by systemic administration of kainic acid. It has also been found that slices obtained from the ventral portion of the hippocampus have a lower threshold for epileptiform bursting induced by an opioid mu receptor than slices from the dorsal end. Thus, these studies clearly demonstrate differences between the ventral and dorsal portions of the hippocampus. Current studies are examining (1) the changes in opioid receptors induced by stimulation of the perforant path and (2) changes in enkephalin and dynorphin levels in the hippocampus as a result of destruction of the DGCs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 90049-06 LMIN
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (Title must fit on one line between the borders) Regulation of Gene Expression in Adrenomedullary Chromaffin Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	J. S. Hong	Supervisory Pharmacologist LMIN NIEHS
Others:	M. K. Stachowiak	Research Scientist Barrow Neurolog Inst
	P. Hudson	Biologist LMIN NIEHS
	R. Tuominen	Assistant Professor Univ. Helsinki
	H. Ye	Research Associate UNC
	M. McMillian	Senior Staff Fellow LMIN NIEHS
COOPERATING UNITS (if any) 		
LAB/BRANCH Laboratory of Molecular and Integrative Neuroscience		
SECTION Neuropharmacology Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER
2.5	2.0	0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK Previous results from our laboratory suggest that long-term treatment of primary cultured bovine adrenal medullary (BAM) chromaffin cells with nicotine or 12-O-tetradecanoyl-phorbol-13 acetate (TPA), which directly activates protein kinase C (PKC), increases the mRNA levels encoding catecholamine synthesizing enzymes and proenkephalin. In the present study, we have examined the effects of nicotine on BAM cell PKC activity with special emphasis on long-term effects. Nicotine increased particulate PKC activity in a concentration dependent manner when measured using in vitro enzyme assay with histone as substrate. This effect is mediated through nicotine cholinergic receptors since 1,1-dimethyl-phenyl-piperazinium (DMPP), a nicotinic agonist, had a similar effect. In addition, chlorisondamine, a specific nicotine receptor blocking drug, antagonized the effect of nicotine. Nicotine also increased specific [3H]PdBu binding within one minute, the effect of which was maximal between 3 and 12 minutes. This effect was similarly reversed by chlorisondamine after 12 min and 18 hrs of nicotine treatment, indicating that continual nicotinic receptor occupancy is required for persistent PKC activation. Compared to PKC activation, the onset of nicotine stimulated diacylglycerol (DAG) production was slow, and it was observed after 12 min of incubation with nicotine. The DAG levels, specific [3H]PdBu binding, and PKC activity remained significantly elevated for at least 18 hrs with continuous nicotine incubation. Furthermore, nicotine increased the PKC immunoreactivity of a particulate protein with a molecular weight of 82kD in the Western blot. These results suggest that nicotine activation increases particulate PKC activity and immunoreactivity in BAM cells. The long-term PKC activation may serve several functions, such as activation of mRNA production and a negative feedback regulation of either nicotinic receptors or voltage dependent Ca⁺⁺-channels.		

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 ES 90053-05 LMN

PERIOD COVERED

October 1, 1991 to September 30, 1992

(Title must fit on one line between the borders)

Neuropeptides: Molecular Mechanism of Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Lawrence H. Lazarus Research Chemist LMN NIEHS

Others: William E. Wilson Research Chemist LMN NIEHS
L. Martti Attila Visiting Fellow LMN NIEHS
Sharon Bryant Bio Science Lab Tech LMN NIEHS

COOPERATING UNITS *(if any)*

S. Salvadori, Ph.D., University of Ferrara, Ferrara, Italy; A Fasolo, M.D., University of Turin, Torino, Italy; L. Pedersen, Ph.D., University of North Carolina, Chapel Hill, North Carolina

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Peptide Neurochemistry Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.5

OTHER

0.5

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

Investigations on the properties of the high delta and mu affinities and selectivities of deltorphins and dermorphin, respectively, was undertaken with nearly 150 synthetic analogues to analyze the interaction of opioids with brain receptors. Our data with deltorphins A, B, and C underscored the following conclusions concerning the requirement for delta affinity and selectivity: (1) reversal of selectivity occurred upon the stepwise N-terminal shift of the C-terminal Asp residue; (2) the lipophilicity of the side-chain of the amino acid residue at position 6 had little bearing on receptor interaction; (3) bulky protective groups on residues 4 or 7, or both, diminished binding to delta receptors; (4) para halogenation of the benzyl ring of phenylalanine enhanced both brain delta receptor binding and bioactivity as measured by use of pharmacological paradigms, however, but not the same halogen for both assays, which further serves to demonstrate differences between central and peripheral opioid receptors; on the other hand, amino or nitro derivatives were detrimental for binding and an anomalous correlation of receptor mu affinity to the lipophilicity and GPI bioactivity; (5) substitutions of phenylalanine suggested that the delta receptor is amenable to ligands in which the aromatic-benzyl ring lies perpendicular to, but not in, the plane of the peptide backbone; (6) molecules extended from the N-terminus or bridged between residue 1-4 exhibited reduced selectivity, yet provided evidence that the delta receptor can accommodate a significantly modified tyrosine residue. Data with dermorphins opened new vistas for the production of super agonists with enhanced mu binding activity. Preliminary computer modeling of the deltorphins tends to confirm the nuclear magnetic resonance analyses that these peptides exhibit a degree of structural integrity in solution; i.e., the N-terminal region forms and type II beta-turn, in which D-alanine and phenylalanine are critical residues and the formation of a folded structure occurs through internal hydrogen bonds to assist in stabilization of its flexible nature. Down regulation of opioid receptors in neuroblastoma cell cultures suggested that G-proteins and unidentified factors are responsible for the modulatory response that may be involved in drug addiction.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 ES 90054-05 LMN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)

Regulation of Biosynthesis, Processing and Secretion of Neuropeptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William C. Wetzel	Senior Staff Fellow	LMN	NIEHS
Others:	Andres Negro-Vilar	Research Physiologist	LMN	NIEHS
	Hector Rivera	Biological Lab Technician	LMN	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Neuroendocrinology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.1

PROFESSIONAL:

1.0

OTHER

1.1

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

Many environmental stimuli influence endocrine and neural homeostasis. These effects may be manifested by changes in chemical communication in these systems, particularly as they relate to reproduction. Since peptides convey much of this information and protein kinase C (PKC) is a major second messenger in these systems, in vivo and cell culture studies have been used to examine the role that PKC plays in pro-LHRH gene expression, peptide biosynthesis, processing and secretion. In rat hypothalamus and an immortalized LHRH neuronal cell line, the LHRH precursor is processed to several different peptides. These studies and Northern blots suggest that prohormone convertase (PC), carboxypeptidase, glutamyl cyclase, and alpha-amidating enzyme may catalyze the reactions. In order to test the role of the PCs more directly, BSC-40 kidney cells were infected with vaccinia virus containing LHRH or in combination with PC1, PC2 or furin. Biochemical studies indicate that pro-LHRH is most efficiently cleaved by PC2. Hence, these studies and Northern blots suggest that PC2 may be the first enzyme in the pro-LHRH metabolic cascade. In secretion studies, the immortalized LHRH neurons secrete LHRH in a pulsatile fashion on the same time-scale as that found in vivo. Since synaptic and gap-junctional connections are formed among these cells, the observed rhythmic behavior may be located within this neuronal network. The immortalized cells appear to secrete LHRH by both the constitutive and regulated pathways. Agents which activate protein kinase C and A used the regulated pathway. Chronic administration of phorbol esters downregulates PKC-alpha, -beta I, -beta II, -delta, -epsilon, but not -zeta in these neurons. These agents also profoundly depress LHRH gene expression. In an attempt to functionally characterize the PKC isoenzymes, we have shown that some of these isoforms show heterogeneity of size and of tissue and cellular distribution. These changes are particularly marked in brain. Future studies will attempt to identify which environmental stimuli affect reproduction through alterations both in PKC and in pro-LHRH gene expression, biosynthesis, processing and secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 ES 90055-04 LMN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)

Hypothalamic Control of the Anterior Pituitary. Morphological Aspects

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Istvan Merchenthaler	Visiting Scientist	LMN	NIEHS
Others:	Andres Negro-Vilar	Research Physiologist	LMN	NIEHS
	Francisco J. Lopez	Visiting Scientist	LMN	NIEHS
	Graziano Ceresini	Guest Worker	Univ. of Parma, Italy	
	David Lennard	Biologist	LMN	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Functional Morphology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

3.3

PROFESSIONAL:

1.3

OTHER

2.0

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

We have demonstrated that luteinizing hormone-releasing hormone (LHRH) and galanin are colocalized in a subset of hypothalamic LHRH neurons and the degree of colocalization is estrogen-dependent. In the female rat hypothalamus, the number of LHRH cells coexpressing galanin is four to five-fold higher than in the male. Progesterone seems to blunt the effect of estrogen on galanin gene expression, since in pregnant, lactating or old animals the degree of colocalization is similar to that seen in the male animals. Our observations also indicate that galanin can be considered as a marker for sexual differentiation, since in male postnatally-castrated animals, whose hypothalami were masculinized by sexual steroids, estradiol induces galanin gene expression if administered in adulthood. Our functional data indicate that galanin regulates reproductive functions at both the hypothalamic and pituitary levels. We have also shown that high levels of progesterone and/or prolactin induce enkephalin gene expression in dopaminergic neurons of the arcuate nucleus. Enkephalin is not produced by this subset of neurons in intact female or male rats; however, in pregnant, lactating or old animals enkephalin appears in dopaminergic neurons of the arcuate nucleus. Enkephalin, co-produced with dopamine, is probably able to antagonize partially the inhibitory effect of dopamine on prolactin secretion.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

201 ES 90056-04 LMIN

PERIOD COVERED

October 1, 1991 to September 30, 1992

(Title must fit on one line between the borders)

Regulation of Hippocampal Opioid Peptides by Excitatory Amino Acids and Hormones

(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jau-Shyong Hong	Supervisory Pharmacologist	LMIN	NIEHS
Others:	W. Q. Zhang	Visiting Associate	LMIN	NIEHS
	C. L. Mitchell	Pharmacologist	LMIN	NIEHS
	L. Thai	Biologist (Q)	LMIN	NIEHS
	C. W. Xie	Research Associate	Duke University	
	J. Douglass	Assistant Professor	Vollum Institute	

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neuropharmacology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.5

OTHER

0.5

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

Several lines of evidence suggest that endogenous opioid peptides, enkephalin and dynorphin play important roles in modulating the excitability of the hippocampus. However, the information regarding the regulation of these two opioid peptides has been lacking. The purpose of this project was to examine the molecular mechanisms of the expression of enkephalin and dynorphin in the hippocampus by excitatory amino acids or hormones, such as glucocorticoids. The regulation of glucocorticoids on dynorphin (DYN) gene expression in the hippocampus was examined using rats removed of adrenal glands for 7, 30, and 60 days. Peptide content in adrenalectomized hippocampi were determined with radioimmunoassays and immunocytochemistry. The DYN mRNA was measured with northern blot analysis and in situ hybridization. A time-dependent attenuation of hippocampal levels of dynorphin in adrenalectomized rats was found which was accompanied by a comparable decrease in the abundance of prodynorphin. An in situ hybridization analysis revealed that both the number of positively hybridized cells as well as the number of silver grains per cell in the dentate gyrus were decreased after ADX. Supplementation of dexamethasone immediately after surgery could reverse the peptide and message attenuation resulted by adrenalectomy. Histochemical study by thionin counter stain showed that the dentate granule cell layer appeared to be intact. The decrement of dynorphin expression in this system is proposed to be resulted from the removal of glucocorticoid input and not dentate granule cell loss. In contrast to the change of dynorphin, ADX has no effect on the expression on enkephalin gene in the hippocampus. Our study provides first evidence for a differential susceptibility of these two peptides to the removal of the glucocorticoid in the hippocampus. since glucocorticoids may serve as a permissive factor in regulating the expression of dynorphin gene in the hippocampus, this provides strong implication for a potential selective glucocorticoid-dependent component in dynorphin gene expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 90057-04 LMIN
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (Title must fit on one line between the borders) Modulation of Epileptiform Activity by Various Excitatory Amino Acid Inhibitors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Clifford L. Mitchell Pharmacologist LMIN NIEHS OTHERS: Laura M. Grimes Special Volunteer LMIN NIEHS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Molecular and Integrative Neuroscience		
SECTION Neurophysiology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.2	OTHER 0.3
CHECK APPROPRIATE BOXES)		
☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK Considerable work in this laboratory has focused on the role of excitatory amino acids in seizure activity and related sequelae. The major objective of this project is to determine the relative contributions of the kainate, quisqualate and NMDA receptors in the generation of epileptiform activity along the trisynaptic pathway of the hippocampal formation. Sustained stimulation of the perforant path activates this entire pathway. When given subcutaneously, the NMDA antagonist, MK-801, prevents status epilepticus and loss of the CA1 and CA3 pyramidal cells associated with this stimulation. However, paroxysmal shaking of the head, neck and trunk, a phenomenon known as wet dog shaking (WDS) is exacerbated by MK-801. Since WDS are associated with epileptiform activity of the dentate granule cells, these results suggested that NMDA receptors may be of little importance in the generation of epileptiform activity at the perforant path - dentate granule cell synapse. However, NMDA receptors appear to be critical for establishment of status epilepticus and subsequent death of the CA1 and CA3 pyramidal cells. To test this hypothesis, we examined the effect of intrahippocampal injections of a non-specific glutamate receptor agonist, gamma-D-glutamylglycine (gamma-DDG), a selective glutamate AMPA receptor antagonist (CNQX) and a selective glutamate NMDA receptor antagonist (MK-801) on the ability to raise the threshold for WDS. All three compounds were effective. Current studies are examining the effect of these agents on electrical afterdischarges in the hippocampus. We are also mapping, using the induction of the proto-oncogene c-fos, the spread of seizure activity throughout the brain. This is of interest since the expression of c-fos by seizure activity is thought to be associated with activation of NMDA receptors.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 90058-04 LMJN
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (Title must fit on one line between the borders) Role of Excitotoxins on Brain-Endocrine Functions		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Andres Negro-Vilar	Research Physiologist LMJN NIEHS
Others:	F. J. Lopez	Visiting Scientist LMJN NIEHS
	M. Moretto	Visiting Associate LMJN NIEHS
	I. Merchenthaler	Visiting Scientist LMJN NIEHS
	A. O. Donoso	Guest Researcher LINC CONICET
COOPERATING UNITS (if any) Laboratorio de Investigaciones Cerebrales, CONICET, Mendoza, Argentina		
LAB/BRANCH Laboratory of Molecular and Integrative Neuroscience		
SECTION Reproductive Neuroendocrinology Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5
CHECK APPROPRIATE BOXES)		
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither		
☐ (a1) Minors		
☐ (a2) Interviews		
SUMMARY OF WORK Excitatory amino acids (EAA) have recently emerged as a new class of neurotransmitters involved in the control of a wide variety of neurophysiological and neuroendocrinological phenomena. In addition to these actions, EAA are considered as excitotoxins, in view of their ability to induce neuronal death as the result of neuronal hyperstimulation. Gamma-amino-butyric acid (GABA) is the prototype neurotransmitter of the classical inhibitory amino acid system, whereas glutamic acid (GLU) is considered as the major representative of the excitatory amino acid neurotransmitters. Studies were conducted to analyze possible interactions between excitatory and inhibitory amino acids in the mechanism controlling neuropeptide secretion. We have used GABA agonists and antagonists as tools to evaluate the participation of the inhibitory amino acid neurotransmission on GLU-induced LHRH release from arcuate nucleus-median eminence fragments incubated in vitro. GABA-A receptors appear to participate in GLU-evoked LHRH secretion, since bicuculine, a GABA-A receptor antagonist, blocked the stimulatory effects of GLU on LHRH release. Interestingly, GLU-induced LHRH secretion was enhanced by activation of GABA-A receptors by muscimol, a GABA-A receptor agonist, in an additive manner. In contrast, GABA-B receptors seem to negatively modulate the effects of GLU. Baclofen, a GABA-B receptor agonist, blocked GLU-evoked LHRH secretion. This effect was abolished by the GABA-B receptor antagonist phaclofen. In summary, our data clearly indicate that a cross-talk between the excitatory and inhibitory amino acid systems constitutes a physiologically important interactive mode in the regulatory mechanisms controlling LHRH secretion and, thereby, reproductive functions.		

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 ES 90059-03 LMIN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)

Biochemical Pathways Involved in Receptor Regulation of Neurotransmitter Levels

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. McMillian	Senior Staff Fellow	LMIN	NIEHS
Others:	J. S. Hong	Supervisory Pharmacologist	LMIN	NIEHS
	P. M. Hudson	Biologist	LMIN	NIEHS
	K. R. Pennypacker	IRTA Fellow	LMIN	NIEHS
	H. H. Suh	IRTA Fellow	LMIN	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neuropharmacology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.8

PROFESSIONAL:

2.3

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

The purpose of this project is to characterize second messenger pathways involved in receptor regulation of neurotransmitter levels. Previous work from our lab demonstrated that angiotensin II (AII) and nicotine each stimulate enkephalin secretion and gene expression in cultured bovine chromaffin cells. Both agonists increase intracellular CA2+ concentration (Cai) and protein kinase C (PKC) translocation through different mechanisms of action. Nicotine activates cation channels, 1-type Ca2+ channels in particular being important for both Cai and PKC responses; the latter response is long-lived and may contribute to enkephalin gene regulation. AII activates phospholipase C (PLC) leading to mobilization of intracellular CA2+; AII also activates CA2+ influx partly through omega-Cgtx-sensitive channel which is critical for secretory effects. AII is ineffective as a secretagogue after inhibition or activation of K+ channels, suggesting that effects are mediated on Cgtx- and (voltage-)sensitive Ca2+ channels via inhibiting K+ channels. We hope that these agonists will be models for excitatory amino acid agonists in rat brain cell cultures. Cultured striatal cells show PKC responses to cation channel-linked receptors-- NMDA, kainate and AMPA-- and to PLC-linked receptor agonists-- taCPD and dopamine and norepinephrine. Dopamine and AMPA responses are dependent on the age of the rat used. The contribution of these agonists, and the Ca2+ and PKC pathways to enkephalin expression in brain cultures is presently being assessed.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 ES 90060-03 LMN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)

Neurotransmitter Secretion and Gene Expression Regulated by Angiotensin System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Harold H. Suh	IRTA Fellow	LMN	NIEHS
Others:	J. S. Hong	Supervisory Pharmacologist	LMN	NIEHS
	M. McMillian	Senior Staff Fellow	LMN	NIEHS
	P. M. Hudson	Biologist	LMN	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neuropharmacology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.2

PROFESSIONAL:

2.0

OTHER

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

The goal of the proposed study is to understand the long-term effects of nicotine and staurosporine on the release of neurotransmitter and [Met5]enkephalin (ME) and the expression of the proenkephalin A (proENK) gene in bovine adrenal medullary (BAM) cells. The specific goal of this project was to elucidate the mechanisms for (1) the release of ME induced by nicotine and staurosporine, and (2) the regulation of the expression of the proENK mRNA. Using primary cultured bovine adrenal chromaffin cells, we found that nicotine increased the release of ME. The pattern of ME release induced by nicotine was biphasic, short-term secretion followed by long-term secretion. The post-treatment study with cholinergic receptor antagonists, hexamethonium and atropine, revealed that long-term (6-9 hrs) stimulation of nicotinic receptors are required for the long-term secretion of ME and the expression of proENK gene. Nuclear run-on experiments showed that nicotine increased transcriptional rate for proENK gene about 2.5-fold 0.5, 1, 3, 5, and 9 hr after drug treatment, indicating that an increase of proENK gene expression induced by nicotine was mediated by an increase of transcription activity. Currently we are studying the mechanisms involved in short-term and long-term secretion of ME. In addition to the opioid system, we are examining the effects of nicotine on catecholamines secretion, tyrosine hydroxylase (TH) gene expression and TH transcription activity. Long-term treatment of BAM cells with staurosporine, a PKC inhibitor, caused neurite-like growth similar to PMA, a PKC activator. Staurosporine decreased the secretion of ME into the media while it increased intracellular content of ME, its precursor, proenkephalin, and proENK mRNA. Like PMA, staurosporine increased AP-1 DNA binding activity. However, the effects of different PKC inhibitors, such as K252a, H7 and sphingosine, were different from staurosporine's responses. The results indicate that staurosporine produced these long-term effects by mimicking a PKC activator, rather than inhibiting PKC activity. This study will reveal the mechanism underlying the regulation of adrenal medullary function of sympathetic neurotransmission. This project will be terminated after September 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 90061-03 LMIN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)

Molecular Mechanisms of Gene Regulation in Adrenal Chromaffin Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Eng-Chun Mar	IPA	LMIN	NIEHS
Others:	J. S. Hong	Supervisory Pharmacologist	LMIN	NIEHS
	P. M. Hudson	Biologist	LMIN	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neuropharmacology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.7

PROFESSIONAL:

1.2

OTHER

0.5

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

We have been interested in elucidating the cell-specific transcriptional factors that regulate the activation of the proenkephalin (PENK) gene in bovine adrenal medullary chromaffin (BAMC) cells and C-6 rat glioma cells. Previous studies indicate that PKC pathway regulates the expression of PENK in BAMC cells. In order to compare the cell-specific expression of this opioid peptide gene, we have used C-6 glioma cells as a model to compare differences in the cellular factors which are involved in the transcriptional regulation. The expression of proenkephalin (PENK) mRNA in C-6 rat glioma cells was stimulated by norepinephrine (a beta-adrenergic agonist) and markedly enhanced by the addition of dexamethasone (a glucocorticoid agonist) to the culture medium, although no induction of glucocorticoid-response-element (GRE)-binding proteins was detectable. In contrast, the stimulation of PENK mRNA expression was not observed with a protein kinase C activator, 12-tetradecanoyl phorbol 13-acetate (TPA), which stimulated the expression of c-fos and c-jun mRNA and their proto-oncoproteins (c-Fos and c-Jun). In addition, an AP-1 activity was induced by TPA and an induction of a kappaB-like binding activity was found with TPA plus cycloheximide treated cells. Together, it suggests that activation of PENK gene in C-6 cells is mainly mediated through beta-adrenergic agonist-elicited cyclic AMP signal pathway, and induction of AP-1 and kappaB-like binding activities appear not to participate in gene activation. Interestingly, the Western blot data showed no increase in intracellular levels of proenkephalin between control and treated cells. However, a marked increase in immunoreactivities for proenkephalin and its derivative, [Met5]enkephalin was detected in medium and a lesser elevation in cells from modulator-treated cell culture through the time-course. These results indicated that there was an association between an increase in PENK mRNA expression and an elevation of proenkephalins and subsequently, the synthesized proenkephalins were released into the medium. (This project will be terminated after September 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 90062-03 LMIN	
PERIOD COVERED October 1, 1991 to September 30, 1992			
TITLE OF PROJECT (Title must fit on one line between the borders) Hypothalamic Control of Pulsatile Prolactin Secretion			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)			
PI:	Francisco J. Lopez	Visiting Scientist	LMIN NIEHS
Others:	Andres Negro-Vilar	Research Physiologist	LMIN NIEHS
	E. Meade Jr.	Biologist	LMIN NIEHS
	L. E. Fogel	Biological Aid	LMIN NIEHS
COOPERATING UNITS (if any)			
LAB/BRANCH			
Laboratory of Molecular and Integrative Neuroscience			
SECTION			
Reproductive Neuroendocrinology Section			
INSTITUTE AND LOCATION			
NIEHS, NIH, Research Triangle Park, North Carolina 27709			
TOTAL STAFF YEARS:		PROFESSIONAL:	
0.7		0.3	
		OTHER 0.4	
CHECK APPROPRIATE BOXES)			
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither			
☐ (a1) Minors			
☐ (a2) Interviews			
SUMMARY OF WORK			
In previous studies, we have demonstrated that pulsatile PRL secretion depicts a distinct pattern depending on the estrous cycle phase. This pattern is characterized by the presence of big mass PRL pulses which present the highest incidence during the afternoon of estrus. In this phase of the estrous cycle, there is an important steroid imprinting, therefore, it is reasonable to think that the highest incidence of BM PRL pulses may be due to this steroid environment. This possibility was evaluated by analyzing pulsatile PRL secretion in OVX animals after treatment with either estradiol, progesterone or both combined. The results indicate that OVX dramatically reduces the incidence of big mass PRL pulses. In fact, PRL pulsatility in two-week OVX animals is characterized by an almost complete absence of large pulses although the pulsatile nature of PRL secretion was not abolished. Three-day estradiol replacement therapy induces a proestrous-like surge 48 h after estrogen administration and, therefore, an endocrine situation similar to that found in the morning of estrus. In these rats, the incidence of BM PRL pulses is restored to that observed in the morning of estrus. In contrast, progesterone (P4) administration the day prior to the bleeding, which stimulates the proestrous P4 surge, impaired the ability of estradiol in reverting the effects of OVX. These observations clearly suggest that estradiol imprinting may favor the establishment of a discontinuous dopaminergic tone, whereas P4, at least at the doses used in this study, blocks this ability. These studies indicate that the preovulatory estradiol imprinting may set a period of interrupted dopaminergic input leading to the establishment of the pulsatile PRL profile observed during estrus. In addition, the ability of progesterone to abolish estradiol effects suggests that progesterone during diestrus is responsible for the instauration of a period of tonic dopaminergic inhibition, producing, thereby, a profile solely consisting of small mass PRL pulses.			

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 ES 90063-03 LMIN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)

Role of Galanin in the Regulation of Gonadal Functions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Francisco J. Lopez	Visiting Scientist	LMIN	NIEHS
Others:	Andres Negro-Vilar	Research Physiologist	LMIN	NIEHS
	M. Moretto	Visiting Scientist	LMIN	NIEHS
	I. Merchenthaler	Visiting Scientist	LMIN	NIEHS
	E. Meade Jr.	Biologist	LMIN	NIEHS
	M. G. Wisniewski	Biologist	LMIN	NIEHS
	L. E. Fogel	Biological Aid	LMIN	NIEHS

COOPERATING UNITS (if any)

Department of Veterinary Anatomy, Ohio State University, Columbus, Ohio

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Neuroendocrinology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.5

OTHER

0.5

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

Studies from our laboratory have provided the basis for considering galanin (GAL) as a neuroendocrine modulator and/or regulator, which participates in the control of gonadal functions. The actions of GAL on the reproductive axis are exerted by endocrine, autocrine and paracrine mechanisms. During the last year, we have provided compelling evidence for most of these mechanisms as well as established a physiological role for the peptide in the control of reproductive events such as the hormonal surges associated with ovulation. Examples for endocrine, autocrine and paracrine regulation by GAL are: the active secretion of the peptide into the hypophyseal portal circulation, the ability of GAL to regulate its own secretion rate from the arcuate nucleus GALergic network, and the actions of GAL secreted from certain pituitary cells for modulating the secretion of others, respectively. Galanin is able to regulate LHRH secretion from arcuate nucleus-median eminence fragments in vitro and requires intact noradrenergic and prostaglandin systems for expressing its effects. Although this constitutes an important observation, the characteristics of the experimental approach do not allow one to distinguish whether the actions of GAL are exhibited directly on the LHRH neuron or are mediated through an interneuronal system. Recently, we have undertaken this question by evaluating the effects of GAL on LHRH secretion from an immortalized LHRH neuronal line. The preliminary results indicate that the peptide has a direct action on LHRH neurons to enhance their secretory activity. Since GAL is secreted into the portal vasculature, it could act at the level of the gonadotrophs to both stimulate basal secretion and enhance LHRH-induced LH release. However, recent studies have indicated that GAL synthesized within the pituitary contributes to the final gonadotropin output from the pituitary. Using an anti-GAL serum developed in our laboratory, we have been able to show that the blockade of GAL actions in dispersed pituitary cells reduces basal LH secretion. Therefore, GAL is a hypothalamic-hypophysiotropic hormone which regulates, under physiological conditions, LH secretion by acting at the level of the LHRH neuronal system and the pituitary gland as either an endocrine (GAL in portal circulation) or paracrine (intrapituitary GAL) regulator.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 ES 90064-03 LMIN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)

The Endocrinology of Normal and Abnormal Puberty

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Penelope K. Manasco	Senior Staff Fellow	LMIN	NIEHS
Others: Michael D. Culler	Expert	LMIN	NIEHS
Andres Negro-Vilar	Research Physiologist	LMIN	NIEHS
Debra C. Godwin	Research Nurse	PEDS ENDO	UNC
Louis E. Underwood	Head	PEDS ENDO	UNC
Susan Muly	Biologist	LMIN	NIEHS
David M. Umbach	Staff Fellow	SBB	NIEHS

COOPERATING UNITS (if any)

General Clinical Research Center, UNC; Division of Pediatric Endocrinology, UNC

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Neuroendocrinology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.4

PROFESSIONAL:

1.1

OTHER

0.3

CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

During the past year we have studied gonadotropin and inhibin pulsatility in 88 normal pubertal children. We have complete studies of LH, FSH, inhibin and testosterone pulsatility in 10 fully pubesced males and 14 prepubertal males. We have shown that inhibin release is pulsatile, that the mean levels of inhibin increase in males as they progress through puberty. In addition, we have shown that the number of peaks of inhibin in a given sampling period does not increase during puberty though the peak height does increase. This pattern of increase of peak height, but not peak number, is the same as that for FSH. LH and testosterone peak numbers and peak heights increase throughout puberty. We have also shown that inhibin increases in response to an intravenous bolus of LHRH. It also appears that the mean levels of inhibin are different in males and females. We are currently working with the statisticians to develop a method for analyzing coincidence of pulses of the hormones studied. In addition, we have begun to study how inhibin, testosterone and gonadotropin pulsatility changes during the day in a cross-sectional study and how release of these hormones changes in a population of children followed longitudinally throughout puberty. We have begun to study children with abnormalities of puberty, including gynecomastia, delayed puberty and cryptorchidism. We plan to study children with exposures to environmental toxins with estrogenic activity, lead, and estrogen-containing hair products. By the end of the summer, we will have entered ten girls in the evaluation of treatment effectiveness of Depot Lupron. We have begun a collaboration with a pediatric endocrinologist at Walter Reed Army Hospital to study a subset of their patients that were found to have had exposure to estrogens through hair products. In this project, we will also work with Dr. Ken Korach to study this question on a mechanistic level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 90065-03 LMN																								
PERIOD COVERED October 1, 1991 to September 30, 1992																										
TITLE OF PROJECT (Title must fit on one line between the borders) Isolation of a Testis Stimulating Factor in Familial Male Precocious Puberty																										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: Penelope K. Manasco</td> <td style="width: 30%;">Senior Staff Fellow</td> <td style="width: 15%;">LMN</td> <td style="width: 15%;">NIEHS</td> </tr> <tr> <td>Others: Michael D. Culler</td> <td>Expert</td> <td>LMN</td> <td>NIEHS</td> </tr> <tr> <td>Susan Muly</td> <td>Biologist</td> <td>LMN</td> <td>NIEHS</td> </tr> <tr> <td>Louisa Laue</td> <td>Expert</td> <td>DEB</td> <td>NICHD</td> </tr> <tr> <td>Janet Jones</td> <td>Research Nurse</td> <td colspan="2">Clinical Ctr. NIH</td> </tr> <tr> <td>David Klapper</td> <td>Prof., Microbiology & Immunology</td> <td>UNC</td> <td></td> </tr> </table>			PI: Penelope K. Manasco	Senior Staff Fellow	LMN	NIEHS	Others: Michael D. Culler	Expert	LMN	NIEHS	Susan Muly	Biologist	LMN	NIEHS	Louisa Laue	Expert	DEB	NICHD	Janet Jones	Research Nurse	Clinical Ctr. NIH		David Klapper	Prof., Microbiology & Immunology	UNC	
PI: Penelope K. Manasco	Senior Staff Fellow	LMN	NIEHS																							
Others: Michael D. Culler	Expert	LMN	NIEHS																							
Susan Muly	Biologist	LMN	NIEHS																							
Louisa Laue	Expert	DEB	NICHD																							
Janet Jones	Research Nurse	Clinical Ctr. NIH																								
David Klapper	Prof., Microbiology & Immunology	UNC																								
COOPERATING UNITS (if any) Developmental Endocrinology Branch, NICHD; Clinical Center, National Institutes of Health; University of North Carolina																										
LAB/BRANCH Laboratory of Molecular and Integrative Neuroscience																										
SECTION Reproductive Neuroendocrinology Section																										
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																										
TOTAL STAFF YEARS: 1.4	PROFESSIONAL: 0.7	OTHER 0.7																								
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☒ (a1) Minors ☐ (a2) Interviews ☒ (b) Human tissues ☐ (c) Neither																										
SUMMARY OF WORK During the past year, we have further refined the first in vitro bioassay system for qualitative and quantitative analysis of the testis stimulating factor. Until now, the only method for determining the presence of the testis stimulating factor utilized testicular artery and vein catheterization of intact cynomolgus monkeys. Using this bioassay, we have been able to show that this factor acts as a protein in ammonium sulfate precipitation. Using size exclusion chromatography, we can show two peaks of bioactivity which are distinct from LH. We have begun a collaboration with Dr. David Klapper at the University of North Carolina to help us with isolation of this factor. To date, we have used the standard isolation techniques of size, ion exchange, and immunoaffinity chromatography. We hope to have the factor isolated in the next year. In addition, we have further studied the second messenger system used by this factor. We will then develop antibodies to allow us to better measure it and to begin to look for the gene responsible for the factor. This factor may have unique clinical significance because it is able to stimulate virilization as well as spermatogenesis in the absence of gonadotropins. It may further be used as a model of a protein that can significantly affect reproduction and may further elucidate other pathways for reproductive effects of toxins.																										

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 90066-02 LMIN
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (Title must fit on one line between the borders) Regulation of AP1 Transcription Factors and Opioid Peptides in Seizure Paradigms		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: K. R. Pennypacker IRTA Fellow LMIN NIEHS Others: J. S. Hong Supervisory Pharmacologist LMIN NIEHS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Molecular and Integrative Neuroscience		
SECTION Neuropharmacology Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK Modulation of opioid gene expression is preceded by the transcription of the AP-1 transcription factors in the brain. The AP-1 transcription complex is composed of two families of proteins: c-fos and fos-related antigens (fra) and the 3 jun proteins. These proteins form heterodimers which recognize and bind to the AP-1 DNA element in the promotor area of the genes. Through this mechanism, gene transcription is modulated. Both the prodynorphin (pDYN) and the proenkephalin (pENK) genes contain AP-1 elements in their promotor regions, suggesting that these factors regulate these genes. We have been examining whether these transcription factors regulate these peptide genes in the rat brain and adrenal gland. Acute administration of the seizure-inducing drug, kainate, induces AP-1 protein expression and modulates the expression of both pDYN and pENK genes in the rat hippocampus. Using anticonvulsants to block kainate-activated neuronal activity, AP-1 protein expression and DNA binding are both inhibited as well as the increase in opioid gene expression, demonstrating the expression of both opioid gene expression and AP-1 proteins are correlated with neuronal activity. During development, rat pups at any age are susceptible to seizure activity after the administration of pentylenetetrazol or kainate; however, neither the AP-1 proteins nor the opioid genes are induced in the hippocampus until postnatal day 14. Of interest, the postnatal day 7 hippocampus contains a high basal level of AP-1 DNA binding, which seems to be due to the high level of mitotic activity at this point in development. Therefore, there is a development-dependent regulation of the expression of AP-1 proteins and opioid genes in the hippocampus after seizure activity.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 90067-02 LMIN
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (Title must fit on one line between the borders) Receptor Regulation of Rat Enkephalin		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	M. McMillian	Senior Staff Fellow
Others:	P. M. Hudson	Biologist
	M. Taylor	Bio Science Aid
	K. R. Pennypacker	IRTA Fellow
	H. H. Suh	IRTA Fellow
	L. Thai	Biologist (O)
	K. Simmons	Bio Science Aid
	J. S. Hong	Supervisory Pharmacologist
		LMIN NIEHS
		LMIN NIEHS
		LMIN NIEHS
		LMIN NIEHS
		LMIN NIEHS
		LMIN NIEHS
		LMIN NIEHS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Molecular and Integrative Neuroscience		
SECTION Neuropharmacology Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER
2.5	2.0	0.5
CHECK APPROPRIATE BOXES)		
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither		
☐ (a1) Minors		
☐ (a2) Interviews		
SUMMARY OF WORK The purpose of this project is to determine which second messenger pathways are involved in receptor regulation of rat adrenal medulla (and spleen, and ultimately brain) enkephalin levels. Previous work from our lab demonstrated that nicotine stimulates enkephalin gene expression in cultured bovine chromaffin cells. Nicotine increases intracellular Ca ²⁺ concentration (Cai) and protein kinase C (PKC) translocation in these cells, and these pathways stimulate the expression of AP-1 binding proteins (Fras and Juns), which bind to and stimulate transcription of the enkephalin gene in bovine cells. However, AP-1 binding proteins are elevated in rat adrenal medulla, and acute nicotine treatment of rats decreases the AP-1 binding (presumably by Ca ²⁺ increasing phosphatase activity); chronic bidaily nicotine, and also saline injections increased adrenal AP-1 binding. Adrenal enkephalin levels are increased over naive levels by all three treatments, but most markedly by acute, repeated nicotine. The enkephalin promoter contains two additional potential transcription factor sites, the cAMP (and Ca ²⁺) responsive element (CRE), and the NFkB site, which may be more important for the response to nicotine. Glial (and possibly neuronal) enkephalin levels are known to be increased by elevating cAMP, and we have demonstrated a glucocorticoid potentiation of this stimulation. Spleen enkephalin levels appear sensitive to NFkB regulation; we have also observed a surprising sex difference in enkephalin levels in the spleen. We are presently comparing adrenal, spleen and cultured brain cell transcription factor levels and their responses to agents known to elevate enkephalin levels, to determine whether the enkephalin gene is regulated similarly or differently in each of these tissues.		

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 ES 90068-02 LMIN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)

Novel Mammalian Opioid Receptor Ligands

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. E. Wilson Research Chemist LMIN NIEHS

Others: L. H. Lazarus Research Chemist LMIN NIEHS

COOPERATING UNITS (if any)

P. Petrusz, University of North Carolina, Chapel Hill, NC; Members of the Mass Spectrometry Lab, LMB, NIEHS

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Peptide Neurochemistry Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK

Two points are the focus of this effort: (1) The variety of consequences of the mu- and delta-opioid receptor agonists/endogenous ligands within localized regions of the central nervous system could indicate (a) degeneracy of those two receptor subtypes or (b) the existence of endogenous ligands other than enkephalins and endorphins. (2) The peripheral (P-) opioid receptor system which mediates inflammation-associated antinociception appears to represent a unique type of opioid receptor; alternatively, it may actually comprise multiple receptor subtypes. Mor et al. (Paris) obtained an antiserum to dermorphin and used it to address point (1) by using it to recover an undefined material from a HPLC column. Our efforts to try to confirm that report have been hampered due to the absence of a suitable antiserum. Because dermorphin is a D-amino acid-containing peptide, we decided to temporarily expand the scope of the investigation to the more general problem of looking for brain peptides which may contain a D-amino acid. An antiserum to an analogue of NMDA was recently characterized (P. Petrusz et al., UNC-CH) and shown to be capable of recognizing a small D-amino acid-containing peptide; while that peptide material is being purified, we are applying Dr. Petrusz's technique to obtain an antiserum suitable for dermorphin/deltorphin. Groups in Germany and Brazil have reported on the apparently paradoxical sensory pain receptors (point 2). As Ferreira's group (in Sao Paulo) has reported that regulation of cyclic-GMP is a consequence of the transduction phenomena controlled by P-receptor(s), a collaborative effort has been initialized to employ the frog skin opioid peptides in an attempt to develop a better understanding of peripheral opioid receptor characteristics.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 ES 90069-01 LMIN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)

Regulation of Opioid Expression in the Striatum: Molecular Mechanisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	David Bronstein	Senior Staff Fellow	LMIN	NIEHS
Others:	H. Ye	Research Associate	UNC	
	K. R. Pennypacker	IRTA Fellow	LMIN	NIEHS
	J. S. Hong	Supervisory Pharmacologist	LMIN	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neuropharmacology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.3

OTHER

0.2

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

It is well established that dopamine (DA) receptor activation causes marked changes in the release and biosynthesis of opioid peptides such as enkephalin (ENK) and dynorphin (DYN) in the striatum. The major objectives in this project are to: 1) examine the possible role that immediate early gene products (e.g., c-fos and related peptides) may play in mediating DA's actions on opioid peptide expression; and 2) explore how glutamatergic systems (e.g., descending cortico-striatal fibers) may interact with DA in the regulation of opioid peptide biosynthesis. DA-containing neurons with cell bodies in the substantia nigra and nerve terminals in the striatum appear to regulate ENK and DYN expression in opposing fashion, with increased DA activation generally leading to increased DYN and decreased ENK expression and decreased DA, causing decreased DYN and increased ENK expression. One explanation for DA's contrasting effects on DYN and ENK is that DA acts via two different DA receptor subtypes (D1 and D2, respectively), which may in turn be linked to different second and third messenger systems. This hypothesis is supported by recent data where we show that c-fos or fos-related antigens (fra) are co-localized with DYN, but not ENK, in striatal neurons. Daily treatment with the DA receptor agonist, apomorphine, caused similar small increases in DYN- and fos/fra-ir. These data support a role for fos/fra proteins in mediating DA's effects on DYN expression in the striatum. Pretreatment with MK-801, a NMDA glutamate receptor antagonist, abolished the apomorphine-induced increases in DYN- and fos/fra-ir, suggesting that glutamate and DA may converge in regulating DYN expression in the striatum. However, results from other experiments, where DA input to one side of the striatum was removed by unilateral injections of 6-hydroxydopamine (6-OHDA) in the substantia nigra, indicate a more complex relationship between DA, glutamate and fos/fra proteins in regulation of opioid biosynthesis. 6-OHDA caused a diffuse low level induction of fos/fra expression in the striatum, but had little effect on DYN-ir. Repeated apomorphine administration to 6-OHDA-treated animals produced 4-fold increase in DYN-ir as well as intense stimulation of fos/fra expression in the lesioned striatum. In contrast to its effects in non-lesioned animals, MK-801 did not reverse apomorphine's effects on fos/fra expression although it blocked the DYN increase. Factors other than fos/fra appear to be involved in nigrostriatal regulation of DYN (and ENK) expression.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 ES 90070-01 LMIN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (Title must fit on one line between the borders)

Identification of Hypophysiotropic Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Istvan Merchenthaler	Visiting Scientist	LMIN	NIEHS
Others:	David E. Lennard	Biologist	LMIN	NIEHS
	Zsolt Liposits	Visiting Scientist	LMIN	NIEHS

COOPERATING UNITS (if any)

Department of Anatomy, University Medical School, Pecs, Hungary

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Functional Morphology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.6

OTHER

0.4

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK

During the past year, we have continued to identify the hypophysiotropic neurons, i.e., those which have connections with portal capillaries of the median eminence; and are, therefore, directly involved in the regulation of the secretion of the anterior pituitary. To identify these neurons, we conducted retrograde labeling studies in combination with peptide immunocytochemistry. Fluoro-Gold was used as retrograde tracer, and the endogenous peptides were detected by fluorescence immunocytochemistry. Our observations indicate that the majority of thyrotropin-releasing hormone (TRH), enkephalin and corticotropin-releasing hormone (CRH) neurons are located in the paraventricular nucleus, while neurotensin, galanin and dopamine neurons are found in the arcuate nucleus. However, not each peptidergic neuron in these well-defined nuclei is hypophysiotropic. Approximately 30% of these neurons do not project to the median eminence but contact other neurons in the brain where hypophysiotropic factors function as neurotransmitters or neuromodulators. Interestingly, the endocrine milieu affects the connectivity between nerve terminals and capillaries in the median eminence. We have found that in lactating animals more than 90% of all dopaminergic neurons in the arcuate nucleus are connected to the portal system, while in cycling females only 65% of these neurons form contacts with the portal system. The retrograde label Fluoro-Gold provides an electron-dense substance in the cytoplasm providing a suitable technique to study the afferentation of hypophysiotropic neurons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 90071-01 LMIN	
PERIOD COVERED October 1, 1991 to September 30, 1992			
TITLE OF PROJECT (Title must fit on one line between the borders) Connectivity of Hypophysiotropic Neurons			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)			
PI:	Zsolt Liposits	Visiting Scientist	LMIN NIEHS
Others:	Istvan Merchenthaler	Visiting Scientist	LMIN NIEHS
	William C. Wetsel	Senior Staff Fellow	LMIN NIEHS
	Andres Negro-Vilar	Research Physiologist	LMIN NIEHS
	Francisco J. Lopez	Visiting Scientist	LMIN NIEHS
	JoAnne Reid	Biologist	LMIN NIEHS
COOPERATING UNITS (if any) Department of Anatomy, University Medical School, Pecs, Hungary			
LAB/BRANCH Laboratory of Molecular and Integrative Neuroscience			
SECTION Functional Morphology Section			
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709			
TOTAL STAFF YEARS: 1.8		PROFESSIONAL: 0.8	OTHER 1.0
CHECK APPROPRIATE BOXES)			
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither			
☐ (a1) Minors			
☐ (a2) Interviews			
SUMMARY OF WORK The hypophysiotropic neurons form the final common pathway for the regulation of the function of the anterior pituitary and they receive inputs from other neurons through synaptic connections. Blood-born substances from the peripheral endocrine organs may also modify the hypophysiotropic neurons by acting through their specific receptors on hypophysiotropic neurons. Our major goal is to identify these connections and receptors at the light and electron microscopic levels. During the last year we have shown that, within the arcuate nucleus, galanin neurons form an intranuclear circuitry by which galanin regulates its own release via a negative ultrashort-loop feedback mechanism. This observation reinforces the notion that galanin shares with other classic hypophysiotropic hormones the ability to regulate the firing rate of its own neuronal network. We have also used an immortalized LHRH cell line from transgenic mice for these studies. Our electron microscopic analysis indicates that the LHRH cell line has morphological characteristics similar to the in vivo LHRH neurons, and these cells form extensive synaptic connections with each other providing a morphological basis for a synchronized secretory activity.			

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01ES 25020-010 LPP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of the Pulmonary Surfactant System and its Modification by Toxic Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G.E.R. Hook	Research Chemist	LPP, NIEHS
Others:	K. Guzman	Senior Staff Fellow	LPP, NIEHS
	W.E. Bakewell	Graduate Student	LPP, NIEHS
	C. Viviano	Graduate Student	LPP, NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Pulmonary Pathobiology

SECTION

Biochemical Pathology Group

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:	3.75	PROFESSIONAL:	3.75	OTHER:	0
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CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Pulmonary surfactant is a heterogeneous complex consisting of surface active phospholipids and specific proteins synthesized and secreted by Type II cells in the alveoli of the lungs. The major lipid component of surfactant is dipalmitoylphosphatidylcholine and the major protein component is surfactant protein A (SP-A). SP-A is a specific surfactant-associated protein found only in the lungs. The function of DPPC is to lower surface tension at the air/cell interface and the function of SP-A appears to be to assist in this process. Biosynthesis and secretion of these substances by alveolar Type II cells is critical for the stabilization and function of the lungs. Silica dust causes massive increases in the pulmonary content of both surfactant phospholipids and SP-A. We have shown that these increases are due to the activation of some, but not all, Type II cells: activated Type II cells make and secrete surfactant phospholipids and proteins at a rate many times faster than normal Type II cells. To understand how silica dust brings about the activation of Type II cells, we are studying regulation of surfactant protein genes in Type II cells and seeking to identify factors capable of upregulating the transcription of those genes. We have mapped the distribution of SP-A in isolated Type II cells using confocal laser scanning microscopy and found that SP-A is localized mainly in the lamellar bodies. However, we also found SP-A in the nucleus of the Type II cell and associated with the perinuclear membrane. In addition we have shown that extracellular SP-A is taken up by isolated Type II cells and distributed in the cell in a manner consistent with its localization in lysosomes and lamellar bodies. In addition, some of the ingested SP-A appears to be translocated to the perinuclear membrane and into the nucleus itself. We do not know the functions of nuclear SP-A at this time but speculate that it could be involved in regulation of the surfactant system. Stimulation of surfactant production in Type II cells is dependent upon the inflammatory condition generated in the lungs by silica. We hypothesize that the surfactant effects arise as a result of the inflammatory condition and, therefore, cellular factors may be released by inflammatory cells that are capable of regulating surfactant production in alveolar Type II cells. Evidence suggests that interleukin-1 β and tumor necrosis factor- α may be part of this stimulatory process because both mRNAs of both cytokines are elevated within a few hours following exposure of rats to silica.

DEPARTMENT OF HEALTH AND HUMAN SERVICES : PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PHS

Z01ES 25021-09 LPP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Differentiation of Tracheobronchial Epithelial Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A.M. Jetten	Research Biologist	LPP, NIEHS
Others:	T. Vollberg	Senior Staff Fellow	LPP, NIEHS
	C. Nervi	Visiting Fellow	LPP, NIEHS
	N. Saunders	Visiting Fellow	LPP, NIEHS
	M. George	Chemist	LPP, NIEHS
	S. Bernacki	Guest Worker	LPP, NIEHS
	K. Marvin	IRTA Fellow	LPP, NIEHS
	W. Fujimoto	Guest Worker	LPP, NIEHS
	K. Mills	Guest Worker	LPP, NIEHS
	T. Hulse	Visiting Fellow	LPP, NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Pulmonary Pathobiology

SECTION

Cell Biology Group

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS: 10

PROFESSIONAL: 9

OTHER: 1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The focus of the research in the Cell Biology Section is to understand the molecular mechanisms that regulate the differentiation of tracheobronchial and epidermal keratinocytes and in particular the role that retinoids play in this process. Squamous cell differentiation is a multi-stage process. In the first stage, cells undergo irreversible growth arrest. This growth arrest is a requirement for the expression of the squamous cell phenotype (stage 2). A characteristic feature of squamous differentiation is the formation of the crosslinked envelope, a layer of crosslinked protein formed beneath the plasma membrane. Two genes, transglutaminase type I and cornifin, whose products play an important role in the formation of this envelope have been cloned. Cornifin is a new cross-linked envelope protein that is high in glutamine and proline. Cornifin becomes crosslinked into high molecular complexes by transglutaminase. The transglutaminase gene has been cloned and sequenced. Several regions that regulate the transcription of this gene have been identified in its promoter. The expression of squamous cell-specific genes is suppressed by retinoids. This regulation appears to occur at the transcriptional level and be mediated by nuclear retinoic acid receptors. Epidermal keratinocytes express the nuclear retinoic acid receptors RAR α , RAR γ and RXR α . Alterations in the expression of these genes may play a role in the process of carcinogenesis. Many squamous cell carcinomas of the lung appear to be defective in the induction of RAR β by retinoic acid. Another example is promyelocytic leukemia where a translocation involving the transcriptional factor PML and RAR α plays a role in the induction of the malignant phenotype. Our laboratory has shown that the PML/RAR α fusionprotein has a similar affinity for retinoids as RAR α and that it, like RAR α , is largely localized in the nucleus. The PML/RAR α fusionprotein forms high molecular weight complexes with either itself or other nuclear proteins. The latter may be important to leukemogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01ES 25023-09 LPP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular and Molecular Mechanisms of Progression of Transformed RTE Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Nettesheim	Chief	LPP, NIEHS
Others:	P. Ferriola	Senior Staff Fellow	LPP, NIEHS
	L. E. Ostrowski	Senior Staff Fellow	LPP, NIEHS
	J. Rundhaug	IRTA	LPP, NIEHS
	T. Gray	Biologist	LPP, NIEHS
	D. Rusnak	Biologist	LPP, NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Pulmonary Pathobiology

SECTION

Epithelial Carcinogenesis Group

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

6

PROFESSIONAL:

4

OTHER:

2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are exploring the role of transforming growth factors α and β (TGF α , TGF β) in the regulation of growth of normal and transformed airway epithelial cells. Normal rat tracheal epithelial cells (RTE) highly sensitive to the growth inhibitory effects of TGF β treatment early in culture but become nearly unresponsive late in culture. However, studies with TGF β 1 and TGF β 2 neutralizing antibodies indicate that DNA synthesis in both early and late cultures is negatively affected by the autocrine TGF β secretion. Cultured normal as well as transformed RTE cells, express 3 different TGF β 1 transcripts, 2.5kb, 1.9kb and 1.4kb in size, respectively. In contrast, A549 cells, a human lung cancer cell line expresses only the 2.5kb TGF β mRNA. Since the 1.4kb transcript had previously not been described, we decided to investigate its structure, regulation and function. Northern analysis, using TGF β subclones, indicates that the 1.4kb transcript contains the complete TGF β coding sequence. PCR cloning of the 3' end of the molecule suggests that it contains the same sequence as the 2.5kb transcript. The 1.4kb transcript is not detectable on northern blots of intact adult tracheas but is expressed during tracheal regeneration *in vivo*. The small TGF β transcript may be more readily translated and may play a role in regeneration and wound healing.

Normal RTE cells are highly epidermal growth factor (EGF) dependent; in contrast, most transformed cell variants are not. Both normal and transformed RTE cells secrete TGF α , a functional equivalent of EGF, which binds to the EGF receptor. Studies with neutralizing TGF α antibody, with the EGFR tyrosine kinase inhibitor Tyrphostin 8 and with TGF α antisense oligonucleotides indicate that both normal and transformed RTE cells are at least partially dependent on autocrine TGF α for growth. Normal cells drastically down regulate TGF α production in late stages of growth while transformed cells do so only to a small extent. Interestingly, transformed cells show reduced levels of EGFR message in northern analysis but express significantly increased numbers of EGF binding sites at the level of the cell membrane. These studies are being continued since functional abnormalities of the EGFR could play a major role in the EGF independence typical of many transformed RTE cell variants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01ES 25027-09 LPP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Functions of Pulmonary Epithelial Cells and Their Secretions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G.E.R. Hook

Research Chemist

LPP, NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Pulmonary Pathobiology

SECTION

Biochemical Pathology Group

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

.25

PROFESSIONAL:

.25

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The functions of the bronchiolar Clara cell are not known although it is generally believed that the cell is secretory. Using Clara cells isolated from the lungs of rabbits we have shown that the major protein secreted by bronchiolar Clara cells is a low molecular weight protein immunochemically and compositionally similar to uteroglobin, the major secretory protein of the uterine epithelium. In addition, we have identified cells of the bronchiolar and tracheal epithelium that contain the Clara cell secretory protein by using an immunogold procedure. Most of the secretory protein appears to be localized in the secretory granules of Clara cells although substantial amounts are located in the endoplasmic reticulum. The low molecular weight protein appears to consist of three major isoforms that differ in their isoelectric points. These secretory products have been used to study the differentiation of the airway epithelium in rats and rabbits. Differentiation of secretory function occurs postnatally in both species and is associated with the appearance of cytoplasmic granules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01ES 25030-06 LPP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Cellular and Biochemical Mechanisms of Particle-Induced Lung Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A.R. Brody	Research Biologist (Supervisory)	LPP, NIEHS
	V. Kalter	Senior Staff Fellow	LPP, NIEHS
	J. Bonner	Senior Staff Fellow	LPP, NIEHS
	A. Osornio-Vargas	Guest Researcher	LPP, NIEHS
	A. Badgett	Biologist	LPP, NIEHS
	P. Coin	Guest Worker	LPP, NIEHS
	T. Perdue	IRTA Fellow	LPP, NIEHS
	L. Blalock	Predocctoral IRTA	LPP, NIEHS

COOPERATING UNITS (if any)

J. Lasky	Guest Worker	LPP, NIEHS
A. Goodell	IRTA Fellow	LPP, NIEHS

LAB/BRANCH

Laboratory of Pulmonary Pathobiology

SECTION

Pulmonary Pathology Group

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

7

PROFESSIONAL:

5

OTHER:

2

CHECK APPROPRIATE BOXES:

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Research in the pulmonary pathology laboratory has been focused upon the basic biological mechanisms through which inhaled particles cause lung disease. We have developed models of asbestosis and silicosis using rats and mice and have shown that the disease process is initiated at junctions of bronchioles and alveolar ducts. One hour of exposure to chrysotile asbestos is sufficient to cause progressive fibrogenesis at alveolar duct bifurcations. The process is initiated by a complement-dependent chemoattraction of lung macrophages to the sites of particle deposition. The central working hypothesis in our laboratory is that these macrophages synthesize and secrete an array of products which mediate the pathogenesis of lung fibrosis. Studies over the past years have shown that macrophages produce a wide array of arachidonic acid metabolites. These are potent inducers of inflammation and cell migration. Now we are focusing upon a group of cell-derived proteins collectively termed "growth factors". The growth factor which is the most potent inducer of mesenchymal cell (e.g. fibroblast and smooth muscle cell) proliferation is platelet-derived growth factor (PDGF). The most potent stimulator of extracellular matrix production by mesenchymal cells is transforming growth factor beta (TGFβ). We have shown that lung macrophages secrete generous quantities of both of these factors. Thus, we postulate that inhalation of toxic particles activates populations of lung macrophages which produce PDGF and TGFβ, the profibrogenic factors. We now know that along with PDGF, macrophages produce alpha-macroglobulin (α-M), a high molecular weight antiprotease which serves as a specific binding protein for the PDGF and significantly augments its capacity to stimulate fibroblast proliferation. Also new is our finding that macrophage-derived PDGF is a potent chemotactic factor for lung fibroblasts, and this activity is enhanced by the receptor-recognized form of the α-M. In addition, we have shown that exposure of lung fibroblasts to asbestos fibers causes an up-regulation of the gene for the PDGFα-receptor. Studies to establish the mechanism for this gene regulation are ongoing studies using *in situ* hybridization show the expression of PDGF mRNA by lung macrophages, while other experiments are designed to block the biological activity of growth factors *in vivo*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01ES 25032-03 LPP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Regulation of Proliferation and Differentiation in Airway Epithelium

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Nettesheim	Chief	LPP, NIEHS
Others:	S. Randell	Senior Staff Fellow	LPP, NIEHS
	J. Liu	Visiting Fellow	LPP, NIEHS
	V. Godfrey	Biologist	LPP, NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Pulmonary Pathobiology

SECTION

Epithelial Carcinogenesis Group

INSTITUTE AND LOCATION

NIEHS Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS: 4	PROFESSIONAL: 3	OTHER: 1
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CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The pathway of cellular differentiation in the tracheobronchial epithelium is not well understood. Studies were undertaken to elucidate the differentiation potential of different airway epithelial cells. We established a series of markers including cell type specific monoclonal antibodies, antikeratin monoclonal antibodies and lectin binding to phenotype isolated rat tracheal epithelial cells. We isolated basal cells and secretory cells using flow cytometry combined with lectin binding and examined their proliferative and differentiation capacity in an *in vivo* culture model suited to study regeneration of tracheal epithelium. We found that basal cells as well as secretory cells are capable of many rounds of cell replication. Both cell types are capable of reconstituting a complete mucociliary epithelium i.e. secretory cells can give rise to basal cells and basal cells can give rise to secretory cells. Both can generate ciliated progeny. Importantly, both basal cells and secretory cells transdifferentiate to a primitive, poorly differentiated (PD cell) phenotype as they initiate proliferation and generate differentiated offspring. While basal cells and secretory cells follow a similar pathway of dedifferentiation, proliferation and redifferentiation, they have very distinct growth factor and attachment substratum requirements.

An air-liquid interface *in vitro* cell culture system was developed and characterized, which supports complete mucociliary epithelial differentiation of rat tracheal cells and establishment of a polarized epithelium. This culture system is now being used to study regulation of growth factor expression during various stages of mucociliary differentiation and to explore the role of various epithelial extracellular matrix molecules in regeneration and differentiation of airway epithelium.

In collaboration with colleagues at the University of North Carolina at Chapel Hill, studies are being conducted to determine which airway cell types express the cystic fibrosis transmembrane chloride channel regulator. Using different airway cell fractions highly enriched in specific cell types, experiments conducted to date suggest that in rat tracheal epithelium the chloride channel regulator protein is mostly expressed in ciliated cells. Experiments are under way to confirm these findings, which are important for the design of gene therapy in cystic fibrosis patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 70060-19 LRDT																				
PERIOD COVERED October 1, 1991 to September 30, 1992																						
TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.) Developmental Biology/Toxicology of Estrogenic Environmental Chemicals																						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">J. A. McLachlan</td> <td style="width: 40%;">Head, Dev. Endocrinol. Pharmacol.</td> <td style="width: 10%;">LRDT NIEHS</td> </tr> <tr> <td>Others:</td> <td>R. R. Newbold</td> <td>Biologist</td> <td>LRDT NIEHS</td> </tr> <tr> <td></td> <td>D. M. Ignar-Trowbridge</td> <td>Senior Staff Fellow</td> <td>LRDT NIEHS</td> </tr> <tr> <td></td> <td>K. G. Nelson</td> <td>Research Biologist</td> <td>LRDT NIEHS</td> </tr> <tr> <td></td> <td>C. T. Teng</td> <td>Senior Staff Fellow</td> <td>LRDT NIEHS</td> </tr> </table>			PI:	J. A. McLachlan	Head, Dev. Endocrinol. Pharmacol.	LRDT NIEHS	Others:	R. R. Newbold	Biologist	LRDT NIEHS		D. M. Ignar-Trowbridge	Senior Staff Fellow	LRDT NIEHS		K. G. Nelson	Research Biologist	LRDT NIEHS		C. T. Teng	Senior Staff Fellow	LRDT NIEHS
PI:	J. A. McLachlan	Head, Dev. Endocrinol. Pharmacol.	LRDT NIEHS																			
Others:	R. R. Newbold	Biologist	LRDT NIEHS																			
	D. M. Ignar-Trowbridge	Senior Staff Fellow	LRDT NIEHS																			
	K. G. Nelson	Research Biologist	LRDT NIEHS																			
	C. T. Teng	Senior Staff Fellow	LRDT NIEHS																			
COOPERATING UNITS (if any) <table style="width: 100%; border: none;"> <tr> <td style="width: 50%;">Bowman-Gray School of Medicine</td> <td style="width: 50%;">University of North Carolina</td> </tr> <tr> <td>Duke University Medical Center</td> <td>University of Würzburg</td> </tr> <tr> <td>Queen's University</td> <td></td> </tr> </table>			Bowman-Gray School of Medicine	University of North Carolina	Duke University Medical Center	University of Würzburg	Queen's University															
Bowman-Gray School of Medicine	University of North Carolina																					
Duke University Medical Center	University of Würzburg																					
Queen's University																						
LAB/BRANCH Laboratory of Reproductive and Developmental Toxicology																						
SECTION Developmental Endocrinology and Pharmacology																						
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																						
TOTAL STAFF YEARS: 6.5	PROFESSIONAL: 3.5	OTHER: 3.0																				
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																						
SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.) Studies continue to determine the cellular and molecular mechanisms by which naturally occurring and synthetic estrogenic compounds interact with various target tissues such as the developing genital tract and skeletal system to cause permanent alterations in differentiation, including dysmorphology and neoplasia. Diethylstilbestrol (DES) is used as a model estrogenic compound. <i>In vivo</i> studies in which DES exposure occurred during development of the murine genital tract (prenatally and/or neonatally) have shown benign and malignant lesions in both male and female animals in structures derived from the Mullerian duct. The molecular genetic features of the tumors are being studied and compared to those reported in human malignancies. Furthermore, descendants of the tumor-bearing mice are being followed for reproductive assessment and increased susceptibility to tumors. Dysmorphology in the genital tract of mice exposed developmentally to DES includes the persistent gene expression of uterine lactoferrin, TGFα, and EGF which may be preneoplastic markers. Studies on phospholipid turnover have shown further insights into signal transduction mechanisms associated with estrogens. Since EGF stimulates uterine cell division in the developing and mature reproductive tract, growth factors may play a role in growth and differentiation of these tissues. Furthermore, it was shown that estrogen regulates the expression of both EGF and TGFα. EGF reproduced the effect of estrogen on induction of DNA synthesis, lactoferrin mRNA levels, and phosphoinositide metabolism. The estrogen-like effects of EGF in the uterus of the mature mouse and uterine-derived cells in culture were dependent on the classical estrogen receptor. Together, these studies support a complex developmental progression in hormone responsiveness in the female and male genital tract with important clues for the development of neoplasia.																						

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70062-05 LRDT

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Role of Growth Factors in Growth and Differentiation of the Reproductive Tract

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: K. G. Nelson Senior Staff Fellow LRDT NIEHS

Others: J. A. McLachlan Head, Developmental Endocrinology and Pharmacology LRDT NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Developmental Endocrinology and Pharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

3.2

PROFESSIONAL:

1.2

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our goal is to determine the molecular targets of estrogenic chemicals and to establish the mechanisms by which interactions of estrogens with the developing genital tract result in permanently altered growth, differentiation, and neoplasia. Evidence suggest that steroid hormone actions in target tissues is mediated by the induction of peptide growth factors. As a first step in our studies of the reproductive tract, we and others are determining the time-dependent and cell-type expression of peptide growth factors that are associated with normal estrogen-induced proliferation (EGF, TGF α , IGF1, TGF β s, PDGFs). Dysregulation of these growth factors are found following neonatal estrogen treatment which may be instrumental in the establishment of the various reproductive tract lesions. Tumor studies using female mice containing a human TGF α gene has revealed that inappropriate expression of a growth factor increases the susceptibility of the reproductive tract to carcinogenesis. Cell culture studies have demonstrated that estrogen-like phenotype similar to that found in vivo can be induced in uterine cells simply by culturing and suggest that the extracellular environment plays a central role in the induction of tissue-specific gene expression. Because of the established role of Vitamin A in reproduction and in the maintenance of epithelial tissues, we are also investigating whether part of the biological effects of steroid hormones is through the modulation of the retinoid acid receptor pathway. To thoroughly understand the molecular mechanisms that control reproductive function, one must integrate the contribution of numerous factors such as the extracellular environment, inflammatory cells, steroid hormones, the retinoids, peptide growth factors and other endocrine hormones.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70065-16 LRDT

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chemical-Receptor Interactions in Reproduction and Hormonal Toxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. S. Korach	Head, Receptor Biology	LRDT NIEHS
Others:	K. Chae	Research Chemist	LRDT NIEHS
	V. Davis	Senior Staff Fellow	LRDT NIEHS
	S. Migliaccio	Visiting Associate	LRDT NIEHS
	O. Gandini	Visiting Associate	LRDT NIEHS
	J. A. McLachlan	Head, Develop. Endocrinol. and Pharmacol.	LRDT NIEHS

COOPERATING UNITS (if any)

University of Würzburg	Brown University Medical School
Laboratory of Molecular Biophysics, NIEHS	UNC Medical School
Medical Foundation of Buffalo	Duke University Medical School

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Receptor Biology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

9.5

PROFESSIONAL:

5.0

OTHER:

4.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Estrogen hormone action in target tissues of the body involves the interaction of the hormonal substance with a receptor protein. The specificity and responsiveness of tissues to hormonal stimulation are governed in most part by the presence and biochemical action of this receptor protein. Nuclear localization of the receptor protein and its activity was shown to be increased by exposure to growth factors, suggesting a coupling of these two signaling pathways. Investigations of the action of various antiestrogens determined that ICI-164384 is a pure antagonist functioning through a mechanism resulting in a rapid loss of the receptor protein within target tissues. This is a unique mechanism not previously described for other hormonal antagonists. Estrogen has an associated effect on bone homeostasis which has now been shown to occur through a receptor mediated mechanism. Bone cells have extremely low estrogen receptor levels. Two experimental bone cell lines were stably transfected with the estrogen receptor. Estrogen was shown to specifically stimulate exogenous and endogenous genes in the clones and parietal cell lines. Prenatal or neonatal estrogen exposure of mice has shown for the first time a highly sensitive and significant influence on adult bone density.

Estrogen receptor is being expressed in yeast for analysis of the gene transcriptional activity of the protein using a variety of hormonally regulated promoter sequences linked to β -galactosidase reporter genes. Site directed mutagenesis of the ligand binding domain of the receptor protein has identified specific amino acids which influence transactivation activity with no effect on ligand binding. Other mutants are being tested to identify a stereochemical ligand recognition site in the protein which biochemical analysis has shown is critical for ligand high affinity binding and agonist activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70067-09 LRDT

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanism of Steroid Hormone in Sex Organ Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C. T. Teng	Senior Staff Fellow	LRDT NIEHS
Others:	Y. H. Liu	Visiting Associate	LRDT NIEHS
	H. Shigeta	Visiting Scientist	LRDT NIEHS
	N. Y. Yang	Visiting Associate	LRDT NIEHS
	J. A. McLachlan	Head, Developmental Endocrinology and Pharmacology	LRDT NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Developmental Endocrinology and Pharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

4.4

PROFESSIONAL:

3.0

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The estrogen-response module (mERM) of the mouse lactoferrin gene which contains an overlapping COUP transcription factor (COUP-TF) and estrogen receptor (ER) binding element, is responsible for estrogen induction. Mutation and deletion of the COUP-TF binding element or reduction of the endogenous COUP-TF increase mERM estrogen-responsiveness. Likewise, overexpressing the COUP-TF expression vector blocked the estrogen-stimulated response of mERM in transfected cells. The molecular mechanism of this repression is due to the competition between COUP-TF and ER for binding at identical contact sites in the overlapping region of the mERM. There is no evidence of heterodimer formation between these two proteins. Our results indicate that two members of the steroid hormone-receptor superfamily work in concert to modulate lactoferrin gene expression. The 5'-flanking region of the human lactoferrin gene was also isolated from a human placenta genomic library. We sequenced 1294 bp of the 5'-flanking sequence, 70 bp of the first exon and 690 bp of the first intron. A major transcription start site was mapped by primer extension. The region immediately upstream from the transcription initiation site, following the first exon is abundant in G and C nucleotides. The promoter was comprised of a noncanonical TATA box (ATAAA), CAAT-like sequence (CAAC), and SP 1 binding site. A PU box, two half palindromic EREs (GGTCA), and an imperfect ERE overlapping with COUP-TF binding site were clustered within a 300 bp stretch (-465 to -165) of the DNA. The elements were also found in the corresponding positions in the mouse lactoferrin gene. Lactoferrin expression in human endometrium cells was stimulated by estrogen and the COUP/ERE element acted as an enhancer in response to the estrogen stimulation. In vitro DNase I footprinting analysis showed binding of the estrogen-receptor on the imperfect ERE. In contrast to the mouse lactoferrin COUP/ERE element, COUP-TF does not interact with this element. Therefore, the molecular mechanisms of the estrogen action that governs the lactoferrin gene expression are different between mouse and human.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 70076-08 LRDT
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Gene Expression in Spermatogenic Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	E. M. Eddy Head, Gamete Biology	LRDT NIEHS
Others:	J. E. Welch Senior Staff Fellow K. D. Fulcher IRTA Fellow D. A. O'Brien IPA C. Mori Visiting Associate D. O. Bunch NRC Fellow	LRDT NIEHS LRDT NIEHS LRDT NIEHS LRDT NIEHS LRDT NIEHS
COOPERATING UNITS (if any) University of North Carolina, Chapel Hill University of Tsukuba Tufts University Kyoto University Faculty of Medicine		
LAB/BRANCH Laboratory of Reproductive and Developmental Toxicology		
SECTION Gamete Biology		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS: 8.5	PROFESSIONAL: 5.5	OTHER: 3.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Gene expression during spermatogenesis in mammals is being examined to define intrinsic and extrinsic mechanisms regulating development and function of male gametes. Genes being studied are expressed uniquely in spermatogenic cells and/or during specific stages of their development. They code for germ cell-specific isoforms of known proteins and include genes for glycolytic enzymes and proteins involved in cell-cell interaction. For example, cDNAs isolated hybridize with RNAs for glyceraldehyde 3-phosphate dehydrogenase (Gapd-s) and hexokinase expressed only in spermatogenic cells. The Gapd-s gene is expressed after meiosis in haploid spermatids, while expression of the hexokinase gene begins during meiosis in pachytene spermatocytes. Glucose is toxic to isolated spermatids but is required by spermatozoa for in vitro fertilization, indicating that unique regulatory processes occur for glycolysis in spermatogenic cells. In addition, mannose 6-phosphate (M6P) receptors have been identified on spermatogenic cells and shown to bind M6P-bearing ligands produced by Sertoli cells. Multiple transcripts for the cation-dependent M6P receptor in germ cells include 1.2 and 1.4 kb mRNAs not found in somatic cells. They probably arise from alternate poly-adenylation signals, one being used in pachytene spermatocytes and the other in spermatids. Transgenic mice have been produced to examine the role of M6P receptors in targeting of acrosin, a sperm-specific serine protease, to the acrosome. Targeted mutations are also being used to knock out the acrosin gene to examine germ cell development and sperm function when there is a mutation in this gene. These studies also serve as models for the effects of gene mutations in humans caused by environmental agents that can be transmitted through the father to the next generation.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 70077-06 LRDT
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Expression of Heat-Shock Genes in Mouse Spermatogenic Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: E. M. Eddy	Head, Gamete Biology	LRDT NIEHS
Others: M. O. Rosario D. J. Dix	Senior Staff Fellow IRTA Fellow	LRDT NIEHS LRDT NIEHS
COOPERATING UNITS (if any) Division of Toxicology Research and Testing, NIEHS The University of North Carolina, Chapel Hill		
LAB/BRANCH Laboratory of Reproductive and Developmental Toxicology		
SECTION Gamete Biology		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS: 3.5	PROFESSIONAL: 2.5	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The synthesis of heat-shock proteins (hsp) following environmental stress is a highly conserved response and apparently protects cells against acute effects of detrimental conditions. One of the most abundant proteins (P70) in spermatogenic cells is related closely to hsp70, as shown by ATP affinity chromatography purification, immunoblots of two-dimensional protein gels, and peptide mapping. The P70 protein is synthesized during the meiotic phase of spermatogenesis. Although several members of the hsp70 gene family have been sequenced and their mRNA expression characterized, little is known about the proteins encoded by these genes. We developed antisera specific to P70 and hsp70 to investigate the expression and role of these proteins in male germ cells. The antiserum to P70 was also used to select clones from a mouse spermatocyte cDNA library. One of these clones hybridized to a testis-specific, developmentally regulated 3.0 kb mRNA whose expression was not affected by heat. Sequence analysis indicated that the clone has high homology to previously reported genes of the hsp70 family. However, it appears that spermatogenic cells do not produce hsp70 mRNA or protein in response to heat stress. This suggests that P70 gene expression is linked to suppression of the hsp70 gene in spermatogenic cells. However, the process of spermatogenesis is unusually sensitive to elevation of temperature and to many toxic agents and P70 may function in germ cell differentiation rather than to protect against the effects of environmental stress. Although P70 expression may be beneficial to the process of spermatogenesis, this process may also leave male germ cells particularly susceptible to environmental stress. Genomic clones have been isolated for the P70 gene and transgenic mice are being produced to examine the spermatogenic cell-specific regulation of expression of this gene. A construct is also being prepared to mutate the P70 gene by homologous recombination in embryonic stem cells. Mice will be produced without functional P70 genes to test the role of the P70 protein in spermatogenic cell function and differentiation.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80040-09 LRDT

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Developmental Pharmacogenetics of Microsomal Steroid Hydroxylases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Negishi	Head, Pharmacogenetics	LRDT NIEHS
Others:	K. Aida	Visiting Scientist	LRDT NIEHS
	K. Nishio	Visiting Scientist	LRDT NIEHS
	M. Iwasaki	Visiting Associate	LRDT NIEHS
	T. Sueyoshi	Visiting Associate	LRDT NIEHS
	N. Yokomori	Visiting Fellow	LRDT NIEHS

COOPERATING UNITS (if any)

Gameete Biology, LRDT/NIEHS	WHO Cancer Center, Lyon, France
Laboratory of Molecular Carcinogenesis, NIEHS	
INSERM, Montpellier, France	

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Pharmacogenetics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

7.3

PROFESSIONAL:

5.0

OTHER:

2.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

As a family of structurally-related enzymes, P450 exhibits extreme diversity in its activity and also in its gene regulation. We study the structural and molecular basis for the diverse activity and regulation, since the diversity affects the human susceptibility to naturally-occurring and man-made toxins and carcinogens. Using site-directed mutagenesis and heterologous expression system, we find that P450 activity can be altered as small as a single amino acid substitution; the substrate specificity of P4502A5 is converted from coumarin to testosterone and, then, to corticosterone by a single mutation of the residue-209 from Phe to Leu and then to Asn. In addition, the steroid-binding site and configuration in the hydrophobic heme-pocket in P450 is elucidated by incorporating the enzymatic characteristics into the computational graphics using the 3D of bacterial P450cam as the model. Transcriptional regulation by growth hormone (GH) is the principal mechanism of sex-specific expression of steroid hydroxylases P45015 α and P45016 α . Using the strain differences in the expression among the domestic and wild mice, we found a transacting locus which represses the transcription of female-specific P45015 α gene in male mice. Transcriptional promoters are determined in the male-specific P45016 α gene and sex-specific nuclear proteins binding to the promoters are identified. This progress in our research is providing the biological basis for understanding the P450 diversities and their effects on the differences in human susceptibility to environmental chemicals.

DIVISION OF BIOMETRY AND RISK ASSESSMENT

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43010-07 DBRA

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Macromolecular Modeling and Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	David G. Hoel	Director	DBRA/OD	NIEHS
Others:	Marshall W. Anderson	Research Chemist	DBRA/LMT	NIEHS
	Lee G. Pedersen	Research Chemist	DBRA/OD	NIEHS
	Tom Darden	Statistician	DBRA/SBB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Division of Biometry and Risk Assessment

SECTION

Office of the Division Director

INSTITUTE AND LOCATION

NIH, NIEHS, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is concerned with determining theoretical factors involved in mutagenesis and in the initial steps of carcinogenesis. The proliferation of new experimental techniques in genetic engineering is providing innovative pathways for studying the dependence of chemically induced mutational events on DNA sequence. Computer modeling is being used to examine the physical chemical factors contributing to site specificity of DNA damage by chemical agents. The same techniques are also being employed to determine changes in molecular properties of oncogene proteins as a consequence of specific mutations, to determine structural modifications in the HIV-1 protease upon inhibitor binding or crystal solvation and to prepare models of mammalian P450S.

Specifically, computer intensive quantum mechanical calculations are employed to determine the properties of small molecules. These results are then used to parameterize empirical force fields that can in turn be used to model the mechanical properties of large molecules such as meaningful segments of DNA and proteins with molecular mechanics/dynamics and computer graphics. Software development/modifications are effected to model our large molecular systems.

Research issues of ongoing interest include the characterization of local structures of DNA sequences (native and chemically modified) that contain known mutational hotspots from mammalian oncogenes and bacterial systems, the examination of the molecular details of the initial attack by mutational metabolites, sequence dependent DNA bending, understanding of the consequences of single amino acid changes on the function of critical proteins such as the p21 ras oncogene protein, understanding the important determinants in inhibitor binding to HIV-1 protease and modeling mammalian P450S from x-ray and site directed mutagenesis data.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-ES-43002-16 EB
PERIOD COVERED October 1, 1991 - September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Human Exposure to Halogenated Aromatic Compounds		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute) PI: Walter J. Rogan Associate Director for Prevention DBRA NIEHS Others: Beth C. Gladen Statistician SBB NIEHS Mei-Lin Yu IRTA Fellow EB NIEHS		
COOPERATING UNITS (if any) Statistics and Biomathematics Branch, NIEHS; National Cheng Kung University, Taiwan		
LAB/BRANCH Epidemiology Branch		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS: 1.95	PROFESSIONAL: 1.95	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human ☐ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☒ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Polychlorinated biphenyls (PCBs) and the DDT/DDE (DDE is the stored metabolite of DDT) family are toxic, widespread pollutants. Both pass from mother to child through the placenta and by contaminating breast milk. This project includes a study of subjects exposed to low levels of both compounds in North Carolina, a study in Mexico where levels of DDE are two to five times higher, and a study of persons poisoned by PCBs in Taiwan. PCBs and DDE can cause long term induction of drug and steroid metabolizing enzymes in animals. In North Carolina, we are planning to look at onset of puberty in a cohort of children we have followed since 1978. In Mexico, in a preliminary analysis, we found that women who have higher levels of DDE in breast milk have shorter lactations. This is similar to what we found previously in North Carolina, and may be a result of the estrogenicity of o,p-DDE, since estrogens decrease milk volume. In Taiwan, an epidemic of 2000 cases of PCB poisoning occurred in 1979. We are planning a study of mortality in the cohort at twelve years of follow-up. We have also found that the severity of the cognitive delay and behavioral disorder seen in children born to poisoned mothers does not diminish with the amount of time between the poisoning and the child's birth, nor does it decrease as the child gets older.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-43004-14 EB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Environmental Exposures and Chronic Renal and Other Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: Dale P. Sandler

Chief, EMES

EB NIEHS

COOPERATING UNITS (if any)

University of Utah College of Nursing; National Institute for Occupational Safety and Health, Cincinnati Ohio

LAB/BRANCH

Epidemiology Branch

SECTION

Environmental and Molecular Epidemiology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.30

PROFESSIONAL:

0.30

OTHER:

0.00

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Environmental exposures may increase the risk of chronic disease, either directly, or through their interaction with individual characteristics such as hormonal status, immune response, or genetic susceptibility. 1.) Data analysis continues in a study of risk factors for chronic renal disease. In a related pilot study, IgA nephropathy, a possibly immunologically-mediated subgroup of glomerulonephritis, was found to be associated with a previous history of kidney diseases, suggesting the possibility of misclassification of earlier episodes. Alternatively, individuals with previous kidney infections, stones, gout, or acute glomerulonephritis may become more susceptible to reno-toxic or immunologic effects of later exposures. Contrary to expectation, allergies were not associated with IgA nephropathy risk, and an apparent association with frequent colds was found to be due to use of antibiotics rather than colds, per se. Risk for IgA nephropathy was found to be associated with use of artificial sweeteners, especially in carbonated beverages, and with employment in wood products industries, work involving car batteries or radiators, and exposure to turpentine and iron fumes or particles. 2.) A cohort of 1000 women who contributed prospective menstrual and reproductive data since 1935 were traced and interviewed to determine their risk of several diseases. Family history of breast cancer and increased cyclic hormone exposure were associated with increased breast cancer risk. Women with shorter cycles and women with more frequent cycles had the greatest risk of breast cancer. Other well established breast cancer risk factors were confirmed. The risk for other diseases such as colorectal neoplasms and gallbladder disease are being evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-ES-44003-15 EB
PERIOD COVERED October 1, 1991 - September 30, 1992		
TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.) Epidemiologic Study of Reproductive Outcomes and Environmental Exposures		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)		
PI:	Allen J. Wilcox Chief	EB NIEHS
Others:	Donna D. Baird Epidemiologist	EB NIEHS
	Clarice R. Weinberg Mathematical Statistician	SBB NIEHS
	Jennifer M. Ratcliffe Visiting Scientist	EB NIEHS
	Ruth E. Little Epidemiologist	EB NIEHS
	Jack A. Taylor Medical Officer	EB NIEHS
	Paige P. Hornsby Health Scientist	EB NIEHS
COOPERATING UNITS (if any) Statistics and Biomathematics Branch, NIEHS, Laboratory of Reproductive & Developmental Toxicology, NIEHS, Columbia University, University of Chicago, University of Bergen, Norway, Free University of Brussels, Belgium		
LAB/BRANCH Epidemiology Branch		
SECTION Reproductive Epidemiology Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.83	1.75	0.08
CHECK APPROPRIATE BOX(ES) ☒ (a) Human ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☒ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This year we published simplified methods for detecting early pregnancy loss using sensitive hormone assays. Our simplified methods are already being put to use by universities and industry. Our recent studies of specific toxins have focussed on an unexplored biological pathway: prenatal exposure. We completed three studies of lasting effects in the adult linked to early exposures as a fetus. Hypotheses were suggested by animal studies of specific toxins. We found no effect of fetal exposure to mothers' smoking on the sons' later sperm characteristics (although smoking by the sons themselves seemed to decrease sperm quality). In a second study, we found no measurable effect of prenatal DES exposure on intellectual performance. In the third, there was some preliminary evidence of premature menopause among women who were exposed as fetuses to DES, suggesting that DES may be an ovarian toxin. We are incorporating molecular genetic methods into our research in order to explore the role of genetic susceptibility to environmental toxins. This year we showed that a genetic variant of the estrogen receptor (reported to be linked with miscarriage risk) had been incorrectly sequenced by previous investigators; furthermore, we found no association with miscarriage risk. The most promising area for genetic susceptibility is likely to be birth defects. In an analysis of data from the Norwegian national registry we found indirect but strong evidence that each type of birth defect has a distinct set of causes, and that these causes include environmental as well as genetic factors. These results encourage us to pursue genetic susceptibility as a major factor in environmental teratogenicity.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-46002-08 EB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Environmental Exposures and Cancer Risk

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Dale P. Sandler Chief, EMES EB NIEHS

Others: Jack A. Taylor Medical Officer EB NIEHS

COOPERATING UNITS (if any)

Cancer and Leukemia Group B Member Institutions, Roswell Park Memorial Institute, University of North Carolina

LAB/BRANCH

Epidemiology Branch

SECTION

Environmental and Molecular Epidemiology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

.45

PROFESSIONAL:

.45

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Data analysis is continuing in a case-control study of risk factors for the acute leukemias in adults in which patients can be classified according to clonal chromosome characteristics, immunologic phenotype, and other biochemical markers. A total of 658 primary leukemia patients who were enrolled in cancer treatment protocols sponsored by Cancer and Leukemia group B, a cooperative cancer study group, and 636 population controls were interviewed regarding exposure to solvents and chemicals, smoking, irradiation, use of potentially toxic medications, and family medical history. An additional 250 AML patients and 90 myelodysplasia patients were also interviewed for related studies. Living within 5 miles of a factory was found to be associated with a 40% increase in risk for AML and a 70% increase in risk for ALL. Although specific industries appeared to be more strongly associated with leukemia risk than others, no individual hazards were identified. There was a 2-fold risk for leukemia associated with smoking among individuals age 60 and over, among whom smoking could be estimated to account for 30% of leukemia risk. Associations varied with morphology and with cytogenetics, suggesting that examining discrete disease subtypes permit more accurate assessment of risk. Similarly a strong association between occupational exposures and *ras* gene activation in AML underscores the importance of identifying homogeneous subgroups of patients. Evaluation of *ras* mutations in 250 additional AML patients is being completed in order to confirm the association between *ras* and chemical exposure. Additional analyses are focusing on hair dyes and marijuana, both of which appear to be associated with increased risk, and on medical history and use of specific medications. Occupational exposure data are being reviewed and reclassified in order to link specific classes of chemical exposure with potential risk for leukemia subtypes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-ES-4700T-06 EB
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Exposure to Radon and Cancer Risk		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute) PI: Dale P. Sandler Chief, EMES EB NIEHS Others: Clarice R. Weinberg Mathematical Statistician SBB NIEHS		
COOPERATING UNITS (if any) Yale University, University of Utah, University of Minnesota, Children's Cancer Study Group, and Statistics and Biomathematics Branch, NIEHS		
LAB/BRANCH Epidemiology Branch		
SECTION Environmental and Molecular Epidemiology Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS: 0.55	PROFESSIONAL: 0.55	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☒ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) The relationship between cumulative residential exposure to radon and cancer risk is being evaluated in an ongoing collaborative study involving Yale University and the University of Utah. The study is designed to evaluate the potential interaction between radon and cigarette smoke exposure. Approximately half of the expected 1000 smokers and 750 nonsmokers with lung cancer and 2100 population controls from Connecticut, Utah, and Southern Idaho have been enrolled. Detailed residential and exposure histories are being obtained, and radon measurements are being made in past homes of participants using year-long alpha track etch detectors, in order to estimate cumulative radon exposure since birth or age 25 for each subject. Data collection has been completed in a pilot phase intended to evaluate factors such as housing characteristics and geology that "predict" radon levels in homes. These data will be analyzed to develop strategies for dealing with missing radon measurements, an important problem in studies involving lifetime exposure assessment. A companion study in Connecticut involving 125 children with cancer and 125 cancer-free controls designed to evaluate the potential childhood cancer risk associated with residential radon exposure is nearly complete. In a study of childhood leukemia risk associated with residential radon exposure that is being conducted in collaboration with a cooperative childhood cancer treatment group, 250 of 325 leukemia patients or controls from a larger case-control study of risk factors for childhood leukemia were found to be eligible for radon measurement based on residential history. Interviews have been completed and detectors placed for 140 of the eligible subjects.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-47002-06 EB

PERIOD COVERED

October 1, 1992 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Effects of Plant Estrogens in Postmenopausal Women

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Donna D. Baird Epidemiologist EB NIEHS

Others: Allen J. Wilcox Chief EB NIEHS
Clarice R. Weinberg Mathematical Statistician SBB NIEHS
John McLachlan Chief LRDT NIEHS

COOPERATING UNITS (if any)

Laboratory of Reproductive and Development Toxicology; Statistics and Biomathematics Branch, NIEHS

LAB/BRANCH

Epidemiology Branch

SECTION

Reproductive Epidemiology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.55

PROFESSIONAL:

0.55

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Exposure to highly estrogenic substances can disrupt reproduction and increase cancer risk, as well as influence bone metabolism and cardiovascular health. Chemicals that are weakly estrogenic are widespread in the environment, including several pesticides. Health effects of these environmental estrogens are not known. This project is designed to determine whether a test-case exposure to environmental estrogens, i.e., exposure to dietary soybeans (rich in plant estrogens) has the expected estrogenic effects. The experimental study compares women who were randomized to a soy-diet group with women who ate normally. We are looking for changes in LH, FSH, sex hormone binding globulin, apolipoprotein A-I, and vaginal smear cytology. If plant estrogens are found to act estrogenically in postmenopausal women, their effects on health outcomes such as osteoporosis merit study. If not, the antiestrogenic effects of these compounds should be investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-49007-04-EB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Occupational Populations Exposed to Carcinogenic Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Eric S. Johnson Visiting Scientist EB NIEHS

Others: Clarice R. Weinberg Mathematical Statistician SBB NIEHS
George W. Lucier Chief LBRA NIEHS
Beth C. Gladen Mathematical Statistician SBB NIEHS

COOPERATING UNITS (if any)

North Carolina State University, Regional Poultry Research Laboratory US Dept of Agriculture (Michigan), Duke Med Ctr (Durham NC), Center Environmental Health & Injury Control CDC (Atlanta), Lovelace Research Inst Inc (Albuquerque, New Mexico)

LAB/BRANCH

Epidemiology Branch

SECTION

Environmental and Molecular Epidemiology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.1

PROFESSIONAL:

1.1

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The serological study of antibodies to avian leukosis/sarcoma viruses (ALSV) and reticuloendotheliosis viruses (REV), and the in vitro study of the infectivity of REV for human cells have been completed, and the results indicate for the first time that humans/human cells can be infected with these viruses. Experiments for detecting integrated ALSV & REV in human genome are in progress. The results of the case-control study of lung cancer in the meat industry indicate that butchers, meatcutters and persons who worked in the stockyard and killing/dressing areas of abattoirs were at extremely high risk of lung cancer, even after controlling for smoking. Contact with raw meat was also a risk factor. These findings point to viruses such as bovine leukemia virus as possibly responsible. Packing and wrapping of meat was also associated with increased risk. Chemical analysis of urine for muconic acid in supermarket workers in Albuquerque is complete, and statistical analysis is in progress.

Mortality analyses of the Baltimore and Missouri cohorts of workers (N=36,000) in a meatcutter and poultry unions has commenced, as is also analysis of a case-control study of tumors of the hemopoietic/lymphatic systems in the meat industry. Follow-up of over 30,000 poultry workers and controls in a Chicago Pension Fund is in progress, as is also an ecologic study of cancer mortality by cattle, poultry and pig inventory in North Carolina.

Analysis of serum levels of TCDD in Australian sprayers is complete, while analyses of other dioxins and furans are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-49002-04 EB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Epidemiologic Studies of Cancer Susceptibility and Oncogene Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: Jack A. Taylor Medical Officer EB NIEHS

Others: Marshall W. Anderson Chief LMT NIEHS
Mary Watson Biologist LMT NIEHS
Teddy Devereux Biologist LMT NIEHS
Walter Piegorsch Mathematical Statistician SBB NIEHS
Doug Bell Staff Fellow LBRA NIEHS

COOPERATING UNITS (if any)

Lab Molecular Toxicol, Lab Biochemical Risk Analysis & Statist/Biomathematics, NIEHS, Univ NC, Duke Univ, Roswell Pk Mem Inst, Univ Georgia, Fox Chase, Telemark Sentralsjukehus(Norway), Finsen Inst(Denmark), St. Mary's Hosp (Grand Junction, CO)

LAB/BRANCH

Epidemiology Branch

SECTION

Environmental and Molecular Epidemiology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27009

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

One of our goals is to characterize the patterns of genetic damage caused by different environmental exposures in key genes which presumably initiate carcinogenesis. Our studies have focused on two model human tumors with strong environmental determinants: non-small cell lung cancer and transitional cell carcinoma of the bladder. In lung cancer we are assaying for K-ras mutational activation at codons 12, 13, and 61, and deactivation of the tumor suppressor gene p53 at exons 4-9 in a large series of tumors from patients with different etiologic exposures. We are currently completing assays of tumors from uranium miners from the Colorado Plateau with heavy radon exposure. In addition we have collected tumors from people with heavy exposure to asbestos, nickel, and tobacco smoke and we will contrast the patterns of DNA damage found in these patients with that found in radon-induced lung tumors. In bladder cancer we have completed assays for H-ras activation in a series of tumors from people exposed to arylamines, cyclophosphamide, or tobacco smoke and are completing assays on p53. In all cases we have been using PCR on formalin-fixed paraffin-embedded surgical blocks, followed by either oligonucleotide hybridization or SSCP with direct sequencing of positive samples.

We have just completed enrollment in a 400 person case-control study on the genetic and environmental determinants of bladder cancer. A major goal of this study is to identify allelic variants of key candidate genes which increase a person's susceptibility to environmentally-induced bladder cancer. We have been genotyping people for two classes of putative susceptibility genes: genes which are responsible for carcinogen metabolism such as N-acetyltransferase, glutathione-S-transferase, and various p450s, and polymorphic proto-oncogenes whose allelic variants may affect susceptibility.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-ES-49003-03 EB
PERIOD COVERED <u>October 1, 1991 - September 30, 1992</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Environmental Effects on Fertility</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)		
PI:	Donna D. Baird	Epidemiologist EB NIEHS
Others:	Allen J. Wilcox	Chief EB NIEHS
	Clarice R. Weinberg	Mathematical Statistician SBB NIEHS
	Andrew S. Rowland	IRTA Fellow EB NIEHS
COOPERATING UNITS (if any) Statistics and Biomathematics Branch, NIEHS		
LAB/BRANCH <u>Epidemiology Branch</u>		
SECTION <u>Reproductive Epidemiology Section</u>		
INSTITUTE AND LOCATION <u>NIEHS, NIH, Research Triangle Park, North Carolina 27709</u>		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.75	1.75	0.00
CHECK APPROPRIATE BOX(ES) ☒ (a) Human ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☒ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The primary purpose of the project on fertility is to develop methods for identifying reproductive hazards. Ultimately, we are working toward developing methods for studying reproductive hazards in small populations so that localized exposures can be evaluated. First, we have developed questionnaire methods for measuring fertility using data on time to pregnancy, the number of noncontracepting menstrual cycles required to conceive. This measure can be the focus of epidemiologic studies of fertility or can be integrated into epidemiologic studies of other diseases such as breast cancer. We applied this method in a study of mercury vapor and nitrous oxide exposures in dental assistants. In addition to studying the effect of these exposures on fertility, we evaluated the accuracy of time-to-pregnancy data from a short, self-administered questionnaire. We are also using this approach to study the effects of prenatal exposure to DES. We will use the DES study to examine the quality of time-to-pregnancy data provided by male respondents. Our second approach focusses on developing biological markers of impaired ovarian function. We have begun by examining urinary metabolites of estrogen and progesterone. These have been measured in daily urine specimens from over 500 menstrual cycles from about 200 women. First, we developed a method for detecting day of ovulation using these urinary hormone data. Next we will develop methods for characterizing follicular development and luteal function.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-ES-49005-01 EB
PERIOD COVERED <u>October 1, 1991 - September 30, 1992</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Lead and Other Neurotoxins as Risk Factors for Amyotrophic Lateral Sclerosis</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)		
PI:	Freja Kamel	Senior Staff Fellow EB NIEHS
Others:	Dale P. Sandler David M. Umbach	Chief, EMES Staff Fellow EB NIEHS SBB NIEHS
COOPERATING UNITS (if any) Statistics and Biomathematics Branch, NIEHS; New England Medical Center, Boston MA; Harvard Medical School, Boston, MA		
LAB/BRANCH <u>Epidemiology Branch</u>		
SECTION <u>Environmental and Molecular Epidemiology Section</u>		
INSTITUTE AND LOCATION <u>NIEHS, NIH, Research Triangle Park, North Carolina 27709</u>		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.15	1.00	0.15
CHECK APPROPRIATE BOX(ES) ☒ (a) Human ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☒ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) Exposure to environmental neurotoxins is likely to increase the risk of developing neurodegenerative diseases, either directly or through interaction with genetic susceptibility factors. Work has begun to assess the role of environmental exposures in the etiology of these disorders. ALS is a progressive neurological disease affecting motor neurons; its etiology is essentially unknown. A protocol has been developed for a case-control study of the role of environmental risk factors in the etiology of ALS. The primary hypothesis is that cumulative lifetime exposure to lead is a risk factor for ALS. Lead exposure will be assessed by measuring bone lead, using the newly developed technology of x-ray fluorescence. Other environmental risk factors which will be investigated include aluminum, mercury, solvents, and pesticides. Biochemical variables reflecting suspected pathogenic mechanisms will also be measured, and their interactions with exposure to lead and other neurotoxins evaluated. The study will involve interviews and clinical and laboratory assessments for 200 incident ALS patients, 200 clinical controls, and 200 population controls.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201-ES-49006-01 EB
PERIOD COVERED October 1, 1991 - September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Paternally-mediated Effects on Reproduction</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)		
PI:	Ruth E. Little Epidemiologist	EB NIEHS
Others:	Jennifer Ratcliffe Visiting Scientist	EB NIEHS
	Kristi-Anne Tolo IRTA	EB NIEHS
COOPERATING UNITS (if any) Institute of Child Health, University of Bristol, Bristol England		
LAB/BRANCH <u>Epidemiology Branch</u>		
SECTION <u>Reproductive Epidemiology Section</u>		
INSTITUTE AND LOCATION <u>NIEHS, NIH, Research Triangle Park, North Carolina 27709</u>		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.75	1.50	0.25
CHECK APPROPRIATE BOX(ES) ☒ (a) Human ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Historically, research on genetic and environmental factors affecting reproduction and subsequent development of the offspring has been centered on the female. Only recently have we come to appreciate the extent and complexity of the role that the male may play. In animals, there is substantial body of experimental evidence indicating that treatment of males with toxicants, followed by mating to untreated females, can results in a variety of adverse reproductive outcomes. These adverse effects on offspring cannot necessarily be predicted by the usual tests of male reproductive function, for these may be normal while the offspring are malformed or die. Only a handful of studies have addressed paternally mediated adverse reproductive outcomes in humans. These are typically marked by inadequate data, narrow exposure focus, and methodologic problems. During this year we have surveyed the world literature, both animal and human, to determine the advisability and feasibility of conducting a broader and more rigorous study of male-mediated reproductive outcomes in humans. This has provided a comprehensive review and synthesis of the literature that is about to be submitted for publication; an invited presentation of the findings was given at the first professional meeting to be devoted to male-mediated reproductive effects. Based on this work, the need for an epidemiologic study of male-mediated reproductive outcomes was clear. We searched for an appropriate data set that would serve at the basis for such a study, and found it at the University of Bristol, England. We have determined the design and scope of this investigation and moved to secure the data that will be available early in 1993. Work on occupational and environmental hazard classifications is proceeding, and analysis will be underway as soon as the data tapes are secured.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21024-11 LBRA

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Drug Metabolizing Enzymes in Animal Models and Human Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	Joyce Goldstein	Pharmacologist	LBRA	NIEHS
Others:	M. Faletto/G. Ibeanu	Staff Fellows	LBRA	NIEHS
	S. DeMoraís	Visiting Fellow	LBRA	NIEHS
	J. Blaisdell	Biologist	LBRA	NIEHS
	P. Linko	Chemist	LBRA	NIEHS
	H. Schweikl	Visiting Fellow	LBRA	NIEHS
	S. Kitareewan	Visiting Fellow	LBRA	NIEHS
	G. Lucier	Chief	LBRA	NIEHS

COOPERATING UNITS (if any)

Judy Raucy, University of New Mexico; Jerry Lasker, Mt. Sinai School of Medicine
Jack Taylor, Epidemiology Branch, NIEHS; Larry Kaminsky, NY State Dept. of Health
Frederick Guengerich, Vanderbilt University School of Medicine

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Metabolism and Receptors

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

6.5

4.5

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☒ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cytochrome P450s comprise the principle monooxygenase system which metabolizes foreign chemicals. There are genetic polymorphisms in many of these enzymes including the CYP2C subfamily (S-mephenytoin metabolism) in man. We have isolated and characterized cDNAs for all of the known members of the CYP2C subfamily including cDNAs for two new genes (2C18, and 2C19). S-mephenytoin metabolism in human liver correlated highly with 2C18 mRNA ($r = 0.95$) and to a lesser extent with 2C9 ($r = 0.65$), suggesting that one or both of these isozymes are involved in this polymorphism. Six members of the 2C subfamily (2C8, 2C9 (2 alleles), 2C18 (2 alleles), and 2C19) were expressed in a yeast cDNA expression system and the recombinant P450s purified from yeast. 2C18 stereospecifically 4'-hydroxylated S-mephenytoin, while neither 2C8 nor 2C9 metabolized this substrate. In contrast, the 11e359 allele of 2C9 metabolized tolbutamide preferentially, while the substitution of one amino acid (Leu359) essentially abolished activity. These results suggest that 2C18 is important in S-mephenytoin metabolism and 2C9 in tolbutamide metabolism, but that allelic variations may also affect metabolism. The anti-coagulant warfarin was also region and stereospecifically metabolized by this subfamily. 2C9 7-hydroxylated S-warfarin, while 2C18 4-hydroxylated S-warfarin. The 2C cDNAs have been inserted into the AHH/TK- cell line, and future studies will characterize their ability to metabolize premutagens. Site-specific mutagenesis was used to produce known allelic variations of human 2C9. These have been tested for their effects on warfarin metabolism and will be tested for their ability to alter metabolism of S-mephenytoin and tolbutamide. The contribution of these enzymes to benzpyrene metabolism will be addressed in future studies. Upstream regions of 2C18 and 2C9 have been isolated. Future studies will address the role of allelic variations in coding and regulatory regions on the ability of humans to metabolize selected drugs. Other studies are addressing the variability of human 1A1 and 1A2 in human liver.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 46004-08 LBRA

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Receptor Interactions for TCDD and Its Structural Analogs: Species Comparisons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: George Lucier Chief LBRA NIEHS

Others: J. Goldstein Pharmacologist LBRA NIEHS
 G. Clark Staff Fellow LBRA NIEHS
 A. Tritscher Visiting Fellow LBRA NIEHS
 Z. McCoy Bio. Lab. Tech. LBRA NIEHS
 C. Sewall Guest Researcher LBRA NIEHS
 J. Vanden Heuvel Guest Researcher LBRA NIEHS

COOPERATING UNITS (if any)

Experimental Carcinogenesis and Mutagenesis Branch, DTRT; Statistics and Biomathematics Branch, and Laboratory of Molecular Toxicology, DBRA; Environmental Protection Agency, HERI; Purdue University, West Lafayette, Indiana

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Metabolism and Receptors

INSTITUTE AND LOCATION

NIH, NIEHS, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

3.5

PROFESSIONAL:

2.7

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☒ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TCDD and its structural analogs such as the polychlorinated dibenzofurans (PCDFs) appear to exert their effects through a mechanism requiring the Ah receptor. TCDD is a potent hepatocarcinogen in female rats but not male rats. Our studies focused on potential mechanisms for the observed sex specificity in a two-stage model for hepatocarcinogenesis in female rats using diethylnitrosamine (DEN) as the initiating agent and TCDD as the promoting agent. These studies revealed that ovarian hormones were required for the tumor promoting actions of TCDD in rat liver. In contrast, ovarian hormones prevented the tumor promoting actions of TCDD in lung. Dose-response relationships for TCDD effects on a number of parameters have been evaluated including CYP1A1 and IA2 induction, epidermal growth factor receptor, estrogen receptor, cell proliferation, preneoplastic lesions, clinical chemistries, TCDD tissue concentrations, other dioxin responsive genes and possible oncogene activation. These studies have revealed that dose response relationships are different for different parameters. For example, CYP1A1 and IA2 induction occurs at much lower doses (0.1-0.3 ng/kg/day) than effects on cell proliferation or preneoplastic lesions. Mathematical analyses of our dose response data for TCDD's effects on CYP1A1, CYP1A2 and the EGF receptor indicated that the best fit to the data was a linear relationship between liver TCDD concentration and response. Similar conclusions were reached when a mechanistic model for TCDD's effects was used to predict dose response relationships. Dose-response relationships were also evaluated by immunocytochemistry and these studies demonstrated that liver cells were heterogeneous in their response to TCDD. Other studies are comparing rodent and human responses by two approaches (1) in vitro culture of human and rodent lymphocytes with TCDD and (2) comparing rodent responses to human effects in cases where humans have been accidentally exposed to TCDD and/or its structural analogs. These studies indicate that human responses to TCDD are similar to those of rats. Of particular interest was our findings of interindividual variation in concentrations of human Ah receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 46007-02 LBRA

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Antisecretory Drugs on Cellular Processes of Stomach, Liver and Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	Claudia Thompson	Research Biologist	LBRA	NIEHS
	George Lucier	Chief	LBRA	NIEHS
Others:	D. Hoel	Director	DBRA	NIEHS
	Z. McCoy	Bio. Lab. Tech.	LBRA	NIEHS
	C. Miller	Biologist	LBRA	NIEHS
	R. DiAugustine	Research Chemist	LBRA	NIEHS
	J. Goldstein	Pharmacologist	LBRA	NIEHS
	G. Clark	Staff Fellow	LBRA	NIEHS

COOPERATING UNITS (if any)

Epidemiology Branch, DBRA
Experimental Toxicology Branch, DTRT

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Cellular Epidemiology

INSTITUTE AND LOCATION

NIH, NIEHS, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2

PROFESSIONAL:

1.3

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☒ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Research on the effects of anti-ulcer drugs on cellular processes has focused on three issues concerning potential risk from long term use. These include determining, (1) the mechanism for carcinoid tumor formation in rodents by studying the relationships between inhibition of gastric acid secretion, serum gastrin levels and cell proliferation, (2) cytogenetic effects [SCEs and chromosomal aberrations (CA)] of Tagamet (cimetidine), Zantac (ranitidine) and Prilosec (omeprazole) in peripheral human lymphocytes and (3) whether omeprazole induces the CYP1A1 dependent enzyme ethoxyresorufin-o-deethylase (EROD) in rodent and human tissues and the mechanism involved. Omeprazole treatment of rats at a dose of 80 μ mol/kg (100x the human dose) increases plasma gastrin, induces EROD activity in rat small intestine but appears to inhibit this activity in liver. Treatment with ranitidine or cimetidine at the highest dose (100x human prescribed dose) caused transient increases in gastrin with no effect on EROD activity. We found that 30% of humans treated with omeprazole (100x lower dose than given rats) had elevated gastrin the magnitude of the increase similar to that seen in rodents. Analysis of SCEs in human lymphocytes before and after 30 days of treatment showed baseline SCE frequency (day 0) were similar for all groups, but after treatment for 30 days a significant increase was observed for the cimetidine (SCE = 5.94 $\pm$ 0.52, n=14 at p=0.002) and omeprazole (SCE = 6.15 $\pm$ 0.53, n=15 at p=0.0002) groups. The analysis of CAs is currently being performed, but preliminary data suggest a weak marginal effect of omeprazole on CAs. Published reports have demonstrated that humans treated with omeprazole for 5 days induces CYP1A1 activity. We have shown that EROD activity in the human hepatoma cell line HEPG2 is induced approximately 10-fold whereas no effect is seen in rodent derived hepatoma cell lines. We are currently determining if omeprazole can bind the Ah receptor and if there are differences in the interaction of omeprazole with receptor derived from human and rodent liver. Quantitative PCR to analyze CYP1A1 mRNA will be applied to human lymphocytes obtained from individuals that have taken omeprazole for 30 days.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 46008-02 LBRA

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Inherited Cancer Susceptibility and Relation to Genetic Damage

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute)

PI:	Douglas Bell	Staff Fellow	LBRA	NIEHS
	Claudia Thompson	Research Biologist	LBRA	NIEHS
	George Lucier	Chief	LBRA	NIEHS
Others;	C. Miller	Biologist	LBRA	NIEHS
	G. Clark	Staff Fellow	LBRA	NIEHS
	E. Stephens	Bio. Lab. Tech.	LBRA	NIEHS

COOPERATING UNITS (if any)

Columbia University, New York
Epidemiology Branch, DBRA

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Cellular Epidemiology

INSTITUTE AND LOCATION

NIH, NIEHS, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☒ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human genetic polymorphisms in metabolic activation and detoxification pathways are a major source of inter-individual variation in susceptibility to cancer. We have developed polymerase chain reaction-based methods to detect DNA sequence polymorphisms in four genes (glutathione transferase mu [GSTM1], debrisoquine hydroxylase [CYP2D6], aryl hydrocarbon hydroxylase [CYP1A1], n-acetyl transferase [NAT2]) associated with increased risk of cancer. There were significant differences in the frequency of the high risk GSTM1 genotype between Caucasian-American, African-American, Taiwanese, and Finnish populations. In an ongoing case-control study of bladder cancer (n=300), we found a -2-fold increased risk associated with the high risk GSTM1 genotype (OR 1.94 95% CI 1.19-3.2). Risk was highest among heavy cigarette smokers. These findings support a protective role for the GT mu enzyme in bladder cancer, particularly among heavy smokers. The results also suggest a possible relationship between ethnic genotype differences and ethnic tumor incidence differences. In addition, we have genotyped ~400 individuals in control and carcinogen-exposed populations and are analyzing how genotypes modulate the level of DNA damage (DNA adducts, mutation frequency, chromosome damage) in lymphocytes. Analysis of HPRT mutation frequency in smokers indicates that years of smoking, as well as current smoking, influences mutation damage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 48005-05 LBRA

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Mechanisms Related to Risk Factors of Mammary Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Richard DiAugustine Research Chemist LBRA NIEHS

Others: S. Snedeker Senior Staff Fellow LBRA NIEHS
G. Jahnke Senior Staff Fellow LBRA NIEHS
G. Lucier Chief LBRA NIEHS
M. Walker Chemist LBRA NIEHS

COOPERATING UNITS (if any)

Epidemiology Branch, DBRA
University of North Carolina, Chapel Hill, NC
University of South Carolina, Charleston, SC

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Hormones and Cancer

INSTITUTE AND LOCATION

NIH, NIEHS, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.3

OTHER:

.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☒ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cell proliferation stimulated by ovarian steroids may be an important determinant of cancer risk in hormonally responsive tissues, since there is an increased risk of genetic errors occurring during the process of cell division. DNA labeling index (DNA LI) was determined in mammary glands of female 35-day old (n=9) and adult cycling (3 to 8 months old, n=29) CD-1 mice, and was contrasted with the uterine luminal and glandular epithelium labeling pattern in each animal. Vaginal smears were obtained to determine stage and regularity of the estrous cycle. Animals were injected (i.p.) with 5-bromo 2'-deoxyuridine (BrDU) at 50 mg/kg to label nuclei in S-phase, and 2 hours later the fourth mammary gland, a mid-section of the uterine horn and a piece of the duodenum were removed, fixed in ice-cold 4% paraformaldehyde overnight, dehydrated, embedded in paraffin and sectioned. Labeled nuclei were detected immunohistochemically with a rat anti-BrDU monoclonal antibody using the avidin biotin peroxidase method. DNA LI was expressed as the percent of labeled epithelial nuclei. Despite a variable pattern in the labeling of uterine epithelium in the 35-day old animals, all mammary glands examined displayed extensive labeling in the terminal end buds (TEBs) (Mean $\pm$ SEM, 13.1 ± 0.7). Ductal labeling was comparatively low (1.3 ± 0.5) and did not appear to be related to the pattern of uterine epithelial labeling in these sub-adult mice. In contrast, mammary ductal labeling in adult animals was negatively correlated with the combined uterine luminal and glandular epithelial DNA LI ($p < 0.05$, $r = -0.426$). Animals (n=7) with the highest uterine DNA LI (40.2 to 70.8) had low mammary ductal DNA LI (< 1.0), while the 4 animals with the highest mammary ductal DNA LI (4.4 to 10.9) had a uterine DNA LI of < 0.5 . Several animals with high mammary labeling had thin uteri, and were at the end of estrus or in metestrus. The inversely correlated patterns of epithelial DNA LI in uterus and mammary gland supports the concept that in adult animals the hormonal requirement for proliferation in the mammary gland is different from that of the uterus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70069-10 LBRA

PERIOD COVERED

October 1, 1991 to September 30, 1992 TERMINATED 05/92

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

DNA Adducts in Human Lymphocytes and Hormone-Dependent Cancers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	Richard DiAugustine	Research Chemist	LBRA	NIEHS
	George Lucier	Chief	LBRA	NIEHS
Others:	C. Thompson	Research Biologist	LBRA	NIEHS
	G. Jahnke	Staff Fellow	LBRA	NIEHS
	M. Walker	Chemist	LBRA	NIEHS

COOPERATING UNITS (if any)

Washington State University, Pullman Washington
Epidemiology Branch, DBRA

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Hormones and Cancer

INSTITUTE AND LOCATION

NIH, NIEHS, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.6

PROFESSIONAL:

0.8

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☒ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

DNA adduct formation is a common mechanism by which structurally diverse compounds can produce mutations and cancer. The enzymatic addition of $^{32}\text{PO}_4$ to the 5' position of nucleotides in digests of DNA has provided a means to detect preformed lipophilic or bulky adducts on thin-layer maps. This ^{32}P -postlabeling method was previously used in this laboratory to evaluate the profile of DNA adducts in lymphocytes of smokers and nonsmokers. We also recently used this method to measure the formation and persistence of BALB/c mouse mammary gland DNA adducts following treatment with benzo[a]pyrene (B[a]P). In the latter study, mammary glands *in vivo* and *in vitro* formed only one major adduct, 7 β , 8 α -dihydro-9 α 10 α epoxy-7,8,9-10-tetrahydro B[a]P deoxyguanosine-3'- PO_4 . This was in contrast to liver, which formed several major adducts. The adduct profile in glands of nulliparous and parous animals was similar, as was the persistence ($T_{1/2}$ approximately 16 days) of the major adduct when measured over a four week period. The ^{32}P -postlabeling method was also used to assess whether the formation of renal tumors in hamsters treated chronically with estrogens is associated with novel or an increased burden of DNA adducts. It was previously reported that estrogenic compounds induce indirectly novel DNA adducts that might be causally related to development of the renal tumors. The DNA of hamster kidneys treated for five months with several different natural or synthetic estrogens was evaluated for appearance of adducts. Close to a dozen intrinsic adducts were identified on TLC maps, but in repeated trials we could find no significant difference in the adduct pattern exhibited by estrogen-treated and cholesterol-treated (control) animals. On the basis of these results, we cannot confirm that new or enhanced DNA adducts as determined by ^{32}P -postlabeling contribute to tumor formation in hamster kidneys.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201-ES-35005-13 LMT
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mechanisms of Carcinogens - Induced DNA Damage and Cell Transformation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)		
PI:	Marshall Anderson	Research Chemist LMT NIEHS
	Theodora Devereux	Research Biologist LMT NIEHS
Others:	Catherine White	Biologist LMT NIEHS
	Monika Hegi	Visiting Fellow LMT NIEHS
	Mary Watson	Biologist LMT NIEHS
COOPERATING UNITS (if any) Dr. Robert Maronpot, Chemical Pathology Branch, NIEHS Dr. Roger Wiseman, Laboratory of Molecular Carcinogenesis, NIEHS		
LAB/BRANCH Laboratory of Molecular Toxicology		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.2	2.2	2.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human ☒ (b) Human Tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) These studies try to identify critical target genes and alterations in these genes which may be important in chemical carcinogenesis. Certain human tumors and chemically induced tumors in animals have a high frequency of proto-oncogene activation, particularly by point mutation of <u>ras</u> genes, and there is evidence for chemical specificity in the pattern of mutations detected. The specific types of oncogene-activating mutations induced by a chemical carcinogen often agree with what is expected based on the DNA adducts formed by that agent. Even for "non-genotoxic" carcinogens, the patterns of <u>ras</u> gene mutations in tumors can give clues about the mechanism of chemically induced tumorigenesis. We are continuing studies on the role of the K-<u>ras</u> oncogene in susceptibility to pulmonary tumorigenesis in inbred mouse strains. Activation of K-<u>ras</u> by point mutations in chemically induced lung tumors from the resistant C57BL/6 mouse is being related to the persistence of DNA adducts in lungs following treatment with methylating nitrosamines. Mutations are identified by single stranded conformation polymorphism, slot blot oligonucleotide hybridization, restriction fragment length polymorphism and direct sequencing following PCR amplification of <u>ras</u> gene fragments in DNA isolated from the tumors. The nude mouse tumorigenicity assay is also employed to try to detect other transforming genes in tumors lacking activated <u>ras</u> genes. Furthermore, analyses of losses of heterozygosity and microsatellite repeats in spontaneous and chemically induced lung tumors from hybrids between resistant mice are being performed in order to try to identify other susceptibility genes. Also, human lung tumors from certain exposure groups, such as uranium miners, are being analyzed for K-<u>ras</u> and p53 mutations in order to understand mechanisms of carcinogenesis by these exposures. In other studies, activation of <u>ras</u> genes by oxazepam, a widely prescribed tranquilizer, and polybrominated biphenyl (Firemaster), is being investigated in order to help understand potential carcinogenic risk of these compounds to humans.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-ES-46005-08 LMT
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Oncogene Activation in Rodent and Human Tumors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)		
PI:	Steve Reynolds Expert Marshall Anderson Chief Colleen Anna Biologist Greg Solomon Biologist Jonathan Wiest Senior Staff Fellow Lori Terry Biologist Astrid Haugen-Strano Biologist	LMT NIEHS LMT NIEHS LMT NIEHS LMT NIEHS LMT NIEHS LMT NIEHS LMT NIEHS
COOPERATING UNITS (If any) Dr. Maronpot, National Toxicology Program, NIEHS		
LAB/BRANCH Laboratory of Molecular Toxicology		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.5	2.5	4.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human ☒ (b) Human ☐ (c) Neither ☐ (a1) Minors Tissues ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Analysis of protooncogene activation in rodent and human tumors has been the major focus of this project. In a previous study, we detected activated protooncogenes were detected in 12 of 14 lung tumors from smokers. From this study, two non-<u>ras</u> genes are being cloned. Approximately 26 kbp has been cloned from the first non-<u>ras</u> gene, appears to correspond to a novel protooncogene and it has been localized to chromosome 10p by <u>in situ</u> hybridization. The cloned sequences have been used to obtain a cDNA clone that gives tumors in the nude mouse tumorigenicity assay. A single human DNA fragment of 16 kbp has been cloned from the second non-<u>ras</u> gene and used to localize this gene to chromosome 20 by <u>in situ</u> hybridization. An additional set of human pulmonary squamous cell carcinomas have been analyzed for activated protooncogenes using the nude mouse tumorigenicity assay. Second cycle transfections indicated that 5 of 16 of these squamous cell carcinomas contained detectable transforming genes that were not H-, K-, or N-<u>ras</u> or c-<u>raf</u> genes as determined by Southern blot analysis. Rat lung tumors from animals treated with diethylnitrosamine (DEN) and promoted with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) scored positive in the tumorigenicity assay for oncogenes. The analysis of highly conserved DNA fragments that contained rat repetitive sequences suggested that the same gene was activated in each of the adenocarcinomas. To further explore the genetics of the K-<u>ras</u> gene in mouse lung tumor susceptibility, the parental origin of K-<u>ras</u> oncogenes detected in lung tumors from F1 hybrids was determined. K-<u>ras</u> oncogenes were derived from the susceptible A/J parent in 38/40 tumors from C3A hybrid mice and 30/30 tumors from AC3 hybrid mice. The observation that the oncogene in hybrids originates from the susceptible parent suggests that the K-<u>ras</u> gene is directly linked to mouse lung tumor susceptibility. Examination of the promoter regions and the regions surrounding the 37 bp repeat in the C3H strain may enable us to determine the mechanism of allele specific activation of the A/J K-<u>ras</u> gene.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-ES-49007-1 LMT	
PERIOD COVERED October 1, 1991 to September 30, 1992			
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Gene Expression - Tumor Progression and Metastasis			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)			
PI:	Marshall W. Anderson	Chief	LMT NIEHS
	Frederick L. Tyson	Senior Staff Fellow	LMT NIEHS
Others:	Maria E. Pimentel	Biologist	LMT NIEHS
	John T. Otstot	Biologist	LMT NIEHS
	Molly S. Vallant	Biologist	LMT NIEHS
COOPERATING UNITS (if any)			
LAB/BRANCH Laboratory of Molecular Toxicology			
SECTION			
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709			
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:	
3.6	1.2	2.4	
CHECK APPROPRIATE BOX(ES)			
☐ (a) Human ☒ (b) Human ☐ (c) Neither ☐ (a1) Minors Tissues ☐ (a2) Interviews			
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) These studies attempt to examine some of the critical differences in gene expression and altered responsiveness to transcriptional regulatory agents that accompany the progression of benign tumors to metastatic lesions. Biological parameters being studied include gene expression, protein expression, tumorigenicity, metastasis and <u>in vitro</u> invasiveness. In order to identify factors involved in tumor progression we have established epithelial cell lines from murine pulmonary adenocarcinomas. Additionally we have developed allografts from spontaneous and chemical induced primary tumors that have been serially passaged in nude mice. A method of quantitative PCR amplification has been used to analyze messenger RNA levels in cell lines and solid tumors. We have obtained several established human lung adenocarcinoma cell lines and developed a number of adenocarcinoma and squamous cell tumor xenografts in BNX mice to compare with the results observed in murine tumors and cell lines with respect to proteinases and their inhibitors. In addition to proteinases and their inhibitors we are also studying the effects of modulating the expression of the putative metastasis suppressor gene <u>nm23</u>. Expression vectors containing the sense and antisense orientation of the <u>nm23</u> coding region are being transfected into nonmetastatic cell lines that express high levels of <u>nm23</u> message and metastatic cell lines with low <u>nm23</u> expression levels. Responsiveness to growth factors such as TGFβ and modulators of differentiation and cell proliferation such as retinoic acid and Vitamin D₃ are being analyzed in murine lung adenocarcinoma cell lines with respect to anchorage independent growth and <u>in vitro</u> invasiveness. Greater than 30% of all human mammary tumors contain amplified and overexpressed HER2/<u>neu</u> with an additional 25% overexpressing HER2/<u>neu</u> in the absence of amplification. Greater than 80% of all pancreatic tumors contain codon 12 mutations in K-<u>ras</u>. We are generating xenografts in BNX mice from human mammary and pancreatic adenocarcinomas for use in evaluating the efficacy of antisense DNA therapy against growth and metastatic spread.			

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES' 40004-15 SBB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Methods in Epidemiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Clarice Weinberg Mathematical Statistician SBB NIEHS

Other: Beth Gladen Mathematical Statistician SBB NIEHS
Gregg Dinse Mathematical Statistician SBB NIEHS
David Hoel Director, DBRA SBB NIEHS
Dale Sandler Epidemiologist EB NIEHS
Allen Wilcox Epidemiologist EB NIEHS

COOPERATING UNITS (if any)

Epidemiology Methods Section, NCI

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

Statistics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are developing and applying new methodologies to research in epidemiology. This year work has progressed in 6 areas: (1) we have developed analytic methods for handling missing data and for fitting nonmultiplicative models, when analyzing data from case-control studies; (2) we have developed statistical methods for extracting information on disease incidence from data on disease mortality; (3) we have published guidelines for designing efficient and informative studies of early (i.e. unrecognized, but biochemically detectable) pregnancy loss; (4) we have developed extensions of earlier models for the probability of conception as related to timing of intercourse (in relation to ovulation), models which now incorporate effects of reproductive toxins and can offer valuable insight into the mechanisms by which such toxins impair human fertility; (5) we have developed strategies for analyzing retrospective time-to-pregnancy studies when the reproductive toxin of interest may have changed in prevalence over calendar time, producing 'time trend' bias; and (6) we have begun work aimed at improving analytic approaches for case-control studies where "disease" status is based, not on physiology, but on circumstantial evidence involving a history of failures (versus history of successes), such as history of repeated miscarriage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 44002-15 SBB
PERIOD COVERED October 1, 1991 - September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mathematical Modeling of Molecular Phenomena		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute PI: Norman L. Kaplan Research Mathematician SBB NIEHS		
COOPERATING UNITS (if any) Department of Statistics, North Carolina State University Depart. of Ecology and Evolutionary Biology, Univ. of CA, Irvine, Department of Genetics, University of California, Davis, CA		
LAB/BRANCH Statistics and Biomathematics		
SECTION Biomathematics Branch		
INSTITUTE AND LOCATION NIH, NIEHS, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human ☐ (b) Human ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Research has continued on developing methodology for analyzing molecular population genetics data. For rare diseases, disease/marker association studies at the population level (e.g. linkage disequilibrium) require that random samples be drawn from within the disease and normal subpopulations separately in order to obtain sufficient sample sizes. To account for the conditional sampling structure, a modified measure of association was proposed and its theoretical mean and variance studied. It was shown that the coefficient of variation of this measure is large enough so that caution is needed in using this measure to locate disease genes. Using a simulation model, the joint distribution of the measure of association and marker heterozygosity in the disease chromosomes was studied, and the likelihood of the observed values assessed as a function of recombination. A critical assumption of the simulation is that the disease is due to one or at most a few ancestral mutations. The simulations show that for very rare diseases this assumption is not compatible with the observation of a large measure of association and high marker heterozygosity in the disease chromosomes; a case commonly seen in practice. Efforts are currently underway to examine measures of association that use two markers simultaneously. Future research will study the distribution of these measures and determine whether it is feasible to use them to infer if the disease gene is located either between the markers or outside them. Finally, the methods are being generalized to handle microsatellite markers which have multiple allelic forms. Standard asymptotic theory is inappropriate here and so exact tests need to be studied.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 45001-12 SBB
PERIOD COVERED October 1, 1991 - September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Experimental Design and Data Analysis Methodology for Animal Experiments		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)		
PI:	Joseph K. Haseman	Research Mathematical Statistician SBB NIEHS
Others:	Gregg E. Dinse	Mathematical Statistician SBB NIEHS
	Beth Gladen	Mathematical Statistician SBB NIEHS
	Walter W. Piegorsch	Mathematical Statistician SBB NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH Statistics & Biomathematics Branch		
SECTION Statistics Section		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.9	1.9	0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human ☐ (b) Human ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project is concerned with statistical methodology issues in the design, analysis, and interpretation of laboratory animal experiments. One investigation demonstrated that while there is a significant correlation between chemical toxicity (as measured by the maximum tolerated dose) and rodent carcinogenicity, this correlation seems limited primarily to gavage studies. Further, the toxicity of a chemical does not seem to be a reliable predictor of whether or not a chemical will be a rodent carcinogen. Another investigation considered the correlation between site-specific carcinogenic effects for chemicals tested in NTP long term studies in rats and mice. For certain sites (e.g., forestomach) there were striking correlations between sexes and species, i.e., chemicals producing these particular tumors tended to increase these neoplasms in both males and females and in both rats and mice. Overall, a chemical producing a site-specific carcinogenic effect in one sex had approximately a 65% chance of producing that same effect in the other sex; similarly, there was approximately a 36% chance that a chemical producing a carcinogenic effect in one species would produce that same site-specific effect in the other species. Future research related to this project includes an estimation of the frequency of false positive and false negative outcomes in the interpretation of long term rodent carcinogenicity studies.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 ES 48001-05 SBB
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Statistical Analysis of Data from Genotoxicity Experiments</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)		
PI:	Walter W. Piegorsch	Mathematical Statistician SBB NIEHS
Others:	Michael D. Shelby	Supervisory Geneticist EMCB NIEHS
	Jack B. Bishop	Research Geneticist EMCB NIEHS
	Jack A. Taylor	Medical Officer EB NIEHS
	Steven T. Garren	Mathematical Statistician SBB NIEHS
COOPERATING UNITS (if any) Experimental Carcinogenesis and Mutagenesis Branch, DTRT Epidemiology Branch, DBRA		
LAB/BRANCH <u>Statistics and Biomathematics Branch</u>		
SECTION <u>Biomathematics Section</u>		
INSTITUTE AND LOCATION <u>NIEHS, NIH, Research Triangle Park, North Carolina 27709</u>		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.35	1.1	.25
CHECK APPROPRIATE BOX(ES) ☐ (a) Human ☐ (b) Human ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The focus of this project is the development of appropriate statistical methodologies for analysis of molecular genetic data from a variety of biological systems. Investigations continued into the modeling and analysis of data from developmental toxicity experiments, with specific attention directed at the dominant lethal assay in the male mouse. Of interest is identification of the sampling distribution of various endpoints from such experiments. A large control database was assembled, from which sampling characteristics of the endpoints of interest were determined. Statistical methods for analyzing data conforming to these characteristics are of the form of distribution free analyses, and require minimal assumptions on the distribution of the response. Various forms of such analyses were compared to identify which of the methods provide optimal statistical characteristics, such as minimal type I error, and maximal power. Permutation trend tests were seen to be quite stable, and were recommended for use. Investigations also continued into the examination and analysis of genetic susceptibility in animal and human subjects. Statistical models and methods were identified for assessing gene-environment interactions; these include logistic regression, complementary log regression, and other generalized linear models.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES'48002-4 SBB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Models in Toxicology and Biochemistry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	Christopher J. Portier	Mathematical Statistician	SBB	NIHES
Others:	Kristin Meier	Staff Fellow	SBB	NIHES
	Michael Kohn	Expert	SBB	NIHES
	Claire Sherman	Mathematical Statistician	SBB	NIHES
	George Lucier	Research Chemist	LBRA	NIHES

COOPERATING UNITS (if any)

Biometry and Field Studies Br., Nat. Inst. of Neurological Disorders & Stroke
Dept. of Mathematics & Statistics, Miami Univ. of Ohio, Oxford, OH
Dept. of Biostatistics, German Cancer Research Ctr., Heidelberg, Germany

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

Risk Methodology Section

INSTITUTE AND LOCATION

NIH, NIHES, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

3.25

PROFESSIONAL:

2.75

OTHER:

0.50

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

This project is intended to increase our understanding of the use and application of mathematical and statistical models in toxicology and biochemistry and to implement new mathematical models to aid in explaining current research findings. The research effort explores a diverse range of biological areas including carcinogenesis, pharmacology, developmental biology, neurology and immunology. In carcinogenesis; (1) an estimate of carcinogenic potency which adjusts for chemically related changes in survival was evaluated and a modification allowing for nonlinear dose-response was developed; (2) a mathematical model of carcinogenesis which explicitly incorporates DNA repair into the multistage process was applied to initiation-promotion experiments in the skin and liver models; (3) health risks from exposure to 2,3,7,8-TCDD are being explored via several mechanistically-based mathematical models; (4) mechanistic models for ligand-receptor binding were developed and their implications for risk assessment were explored; (5) the shape of the carcinogenesis dose-response curve using information on biological activity of the chemical has been studied and suggests that chemical structure correlates well with dose response shape; (6) a multistage model of carcinogenesis incorporating an explicit component for stem cells was developed and applied to an initiation-promotion study in mouse skin; (7) alternatives to the two-stage model of carcinogenesis utilizing multiple pathways to better incorporate the role of oncogenes are underway; (8) extra-Poisson variability in two-stage models of carcinogenesis is being explored. In teratology, it was found that resampling techniques and quasi-likelihood methods can be used to account for interlitter correlations when analyzing teratological data. In immunotoxicology, it was found that there is little or no relationship between immunotoxicity and mutagenicity but that carcinogenicity and immunotoxicity are related with immunotoxic compounds having a high probability of being carcinogenic. In neurology, a research effort is underway with NINDS to apply multivariate smoothing techniques to characterize the relationship between areas of the brain and different parts of the body. Also, the application of smoothing spline methods to the estimation of rate functions in pharmacology and biochemistry is underway. Finally, a graph theoretical modeling technique for qualitative determination of the regulatory properties of biochemical networks was developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01ES49004-01 SBB
PERIOD COVERED <u>October 1, 1991 - September 30, 1992</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Statistical Methods in Human Development/Clinical Studies</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)		
PI:	David M. Umbach	Staff Fellow SBB NIEHS
Others:	Penelope K. Manasco	Senior Staff Fellow LMIN NIEHS
	Clarice R. Weinberg	Mathematical Statistician SBB NIEHS
	Allen J. Wilcox	Epidemiologist EB NIEHS
COOPERATING UNITS (if any) None		
LAB/BRANCH <u>Statistics and Biomathematics Branch</u>		
SECTION <u>Statistics Section</u>		
INSTITUTE AND LOCATION <u>NIEHS, NIH, Research Triangle Park, North Carolina 27709</u>		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human ☐ (b) Human ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project was initiated to develop new statistical methodologies for problems in human development and to apply existing methodologies to those problems in novel ways. Work has begun in two areas: (1) statistical modeling of serum concentrations of reproductive hormones in peripubertal children and (2) statistical modeling of the distribution of birth weights in human populations. Secretion of pituitary gonadotropins and gonadal hormones is pulsatile; serum profiles exhibit distinct peaks with irregular spacings. With an appropriate statistical model, one could describe these profiles with a few parameters, allowing comparisons among subjects with normal and abnormal pubertal development. Such comparisons would provide insight into mechanisms of dysfunctional development. We are currently evaluating models used for adult hormone profiles to see whether they also provide adequate descriptions in prepubertal children. These models are limited to one hormone at a time; our long term goal is to develop models for suites of hormones as interacting systems. Development of a satisfactory statistical model for the distribution of birth weights in a population will facilitate a refined understanding of the relationship of birthweight to infant mortality. We have developed a model which allows a parsimonious description of a birth weight distribution and which can be used to investigate social and environmental influences on birthweight distributions.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21156-02 DTRT

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular Protein Changes in the Molecular Biology of Cancer.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: James K. Selkirk, Toxicologist (NIEHS\DTRT)

Others: Alex Merrick, Toxicologist (NIEHS\DTRT)

Choaying He, Visiting Associate (NIEHS\DTRT)

Rachel Patterson, Biologist (NIEHS\DTRT)

Lora Long, Biologist (NIEHS\DTRT)

COOPERATING UNITS (if any)

LAB/BRANCH

Division of Toxicology Research & Testing

SECTION

INSTITUTE AND LOCATION

National Institute of Environmental Health Sciences, Res. Tri. Pk., N.C.

TOTAL STAFF YEARS:

4.5

PROFESSIONAL:

2.5

OTHER:

2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are probing the relationship of oncogene activation as the stimulus to the phenotypic expression of transformation. It has become clear in recent years that the mechanism of cancer formation by environmental chemicals involves complex genetic alterations related to the binding of metabolically activated chemical intermediates with cellular macromolecules. While genes are the template for mutation, translation of oncogenes into active proteins are critical for phenotypic expression of mutation and cancer. Since cancer is the somatic expression of a genetic event we would like to understand the cascade of biochemical steps that steer the initiated cell through the malignant transformation process. The advent of multiple gel technology and the concomitant use of computerized image analysis has enabled a more sophisticated approach to the analysis of the enormous amount of bio-molecular information present in 2D Gels for both structural identification and comparative pattern analysis of proteins.

The burgeoning gene sequence databases are a valuable adjunct to our research since it offers the possibility of retracing critical proteins identified from gels back to the gene of origin. This can be accomplished by "backtranslating" genes to determine the triplet code template nucleotide sequence and searching via special algorithms to match the deduced sequence against known genes.

Since the transformed phenotype is the result of aberrant regulatory and/or structural proteins we are advancing into identifying both the presence of oncoproteins and suppressor proteins as a result of chemical treatment as well as the changes in the cellular protein profile as a response to the presence of active oncoproteins.

DIVISION OF TOXICOLOGY RESEARCH AND TESTING

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21098-06 CCB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Adverse Effects of Lindane in B6C3F1 Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: H.L. Hong Biologist CCB NIEHS
Others: G.A. Boorman D.V.M., Ph.D., Chief CCB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Chemical Carcinogenesis Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

0.4

PROFESSIONAL:

0.4

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have shown that 3-day lindane exposure at doses of 20 and 40 mg/Kg/day suppressed bone marrow cellularity, erythrocyte precursors (CFU-E), granulocyte-macrophage progenitor cells (CFU-GM), and residual progenitor cell damage could be demonstrated by whole body irradiation (two WBI 200 rads). Male B6C3F1 mice given lindane daily at doses of 0, 10, or 20 mg/Kg body weight by gavage in corn oil for 10 consecutive days did not show clinical abnormality or changes in body weights, but there was a dose-dependent decrease in marrow cellularity, in more pluripotent stem cells (CFU-S), and in committed CFU-GMs which returned to control values by four weeks. The mice were then subjected to two 100-rad exposures of WBI at four and nine weeks following cessation of lindane treatment. This level of irradiation caused only a transient drop in marrow progenitor numbers. Control and lindane-exposed mice were examined at one and six weeks following the last irradiation, which was 10-15 weeks following the final lindane exposure. The lindane-exposed mice had lower progenitor cell numbers and slower recovery from the irradiation. These results indicate that lindane has significant myelotoxicity in mice and short-term lindane exposure can induce residual progenitor cell damage that can be demonstrated by subsequent irradiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21113-05 CCB

PERIOD COVERED

October 1, 1991 - September 30, 1992 TERMINATED

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Myelotoxicity in Mice Caused by Drinking Mixture of Groundwater Contaminants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	H.L. Hong	Biologist	CCB	NIEHS
Others:	G.A. Boorman	D.V.M., Ph.D., Chief	CCB	NIEHS
	R.S.H. Yang	Ph.D.	STB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Chemical Carcinogenesis Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The possible toxicological interaction of the 25-chemical mixture of groundwater contaminants and whole-body irradiation (WBI) on hematopoiesis was investigated. Exposure of the chemical mixture (5% mixture stock) to B6C3F1 mice further reduced bone marrow stem-cell proliferation following radiation injury resulting from repeated WBI at 200 rads. Even 10 weeks after cessation of chemical-mixture treatment when all hematological parameters were normal, a residual effect of the chemical mixture may still be demonstrated as lower bone marrow stem-cell counts following irradiation. There is also an indication that even at 1% mixture stock level an enhancement of radiation injury to hematopoiesis may be detected if the exposure period is long enough. These examples provide a clue that the toxicology of chemical mixtures at environmental levels will probably not involve acute toxic responses. It is most likely an insidious effect(s) disrupting the homeostasis of the organism. The exposed animals may appear totally 'normal' clinically or based on conventional toxicological endpoints. However, such a subclinical state may provide a basis for enhancement or potentiation of otherwise mild toxic responses from an acute exposure(s) of chemical, physical and/or biological agents. In this sense, the concept of a generic 'promotor' or 'enhancer' for any possible toxicity may be advanced for the potential toxicologic consequence of a mixture of environmental pollutants. These findings also raise the possibility of synergistic interaction between a background long-term, low-level chemical mixture exposure and a subsequent acute dose resulting from accidental exposure or drug intake including alcohol abuse. These results were published in Toxicol. Lett. (1989), 49, 183-197; (1991), 57, 101-111; JEPTO (1992), 11(2), 1-10.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21141-03 CCB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Growth and Tumors of Rats and Mice Fed NIH-07 and NTP-90 Diets

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	G.N. Rao	D.V.M., Ph.D.	CCB	NIEHS
Others:	J. Edmondson	Biologist	CCB	NIEHS

COOPERATING UNITS (if any)

Comparative Medicine Branch

LAB/BRANCH

Chemical Carcinogenesis Branch

SECTION

Laboratory Animal Management

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

1.6

PROFESSIONAL:

0.1

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The maximum mean body weights of rats attained during the course of two-year studies increased by about 20% from 1975 to 1985. Higher body weights will lead to increases in the incidences of mammary tumors, pituitary tumors, and possibly other tumors. Modification of diet may slow the growth and lower the maximum body weight attained which in turn may decrease the incidences of spontaneous tumors. A lower protein diet may decrease the incidence and severity of kidney disease. The purpose of this study is to determine the feasibility of 13% protein and 20% fiber diet (NTP-90) in lowering the maximum body weight and decreasing the severity of nephrosis in rats and mice in comparison with high protein, low fiber NIH-07 diet. Groups of 60 male and 60 female F344 rats and B6C3F1 mice are receiving NIH-07 or NTP-90 diets for two years. Body weights, feed consumption and clinical chemistry parameters are determined at selected intervals. Tissues will be collected for evaluation of nonneoplastic and neoplastic changes at the end of the study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21147-02 CCB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Effect of Sodium Fluoride Administration on Radiation Induced Osteosarcomas

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	H.L. Hong	Biologist	CCB	NIEHS
Others:	R. Irwin	Ph.D.	CCB	NIEHS
	G.A. Boorman	D.V.M., Ph.D., Chief	CCB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Chemical Carcinogenesis Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The NTP has sponsored two sodium fluoride carcinogenicity studies. Groups of F344 rats and B6C3F1 mice received 0, 25, 100 and 175 ppm sodium fluoride (NaF) in their drinking water. An equivocal tumorigenic response occurred in male rats, but no response was observed in female rats or mice. Male rats appear to be more sensitive to the potential carcinogenic effects of NaF than female rats or mice. Since irradiation can be altered to produce various osteosarcoma rates, this follow-up study would be an obvious means of evaluating the possible promoting activity of NaF. The objective is to determine whether radiation induced osteosarcoma in rats is influenced by exposure to NaF in the drinking water. Male F344 rats received 3000 rads of irradiation to the right rear limb with 137 Cesium source. Ten days later, non-irradiated and irradiated rats were given NaF at 0 and 275 ppm in drinking water daily for 2 years. The surviving rats will be evaluated histopathologically.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21166-01 CCB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Growth and Tumors of Rats and Mice Fed NIH-07, NTP-91 and NTP-92 Diets

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	G.N. Rao	D.V.M., Ph.D.	CCB	NIEHS
Others:	J. Edmondson	Biologist	CCB	NIEHS

COOPERATING UNITS (if any)

Comparative Medicine Branch

LAB/BRANCH

Chemical Carcinogenesis Branch

SECTION

Laboratory Animal Management

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

1.4

PROFESSIONAL:

0.1

OTHER:

1.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Fischer 344 (F344) inbred is the selected rat strain for evaluation of toxic and carcinogenic potential of chemicals. Leukemia is the major cause of mortality for the F344 rat, especially the male. The incidence of leukemia in the F344 rat has increased over the last 15 years and the survival/median life span has decreased. Corn oil or safflower oil, when given by gavage, decreased the incidence of leukemia in the males and increased the survival/median life span. The purpose of this study is to determine the influence of 10% fat diets (NTP-91 diet containing corn oil, and NTP-92 diet containing safflower oil) on the incidence of leukemia and survival when fed to F344 rats for two years. Groups of 60M + 60F F344 rats are fed NIH-07 diet, the standard diet with 5% fat, or the NTP-91 or NTP-92 diets for two years. Body weights, feed consumption and clinical chemistry parameters are determined at selected intervals. Tissues will be collected for evaluation of nonneoplastic and neoplastic changes at the end of the study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-ES-21168-01 CCB
PERIOD COVERED <u>October 1, 1991 - September 30, 1992</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Toxicokinetics of Pentachlorophenol in F344 Rats</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)		
PI: J.H. Yuan Others: T.J. Goehl R. Irwin H.L. Hong	Visiting Associate Ph.D. Ph.D. Biologist	CCB CCB CCB CCB NIEHS NIEHS NIEHS NIEHS
COOPERATING UNITS (if any) Comparative Medicine Branch		
LAB/BRANCH Chemical Carcinogenesis Branch		
SECTION		
INSTITUTE AND LOCATION NIEHS, Research Triangle Park, NC 27709		
TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Toxicokinetics of pentachlorophenol (PCP) was studies in male and female F344 rats using iv (5 mg/Kg) and oral gavage (9.5, 38, and 75 mg/Kg) routes. After a bolus iv dose of 5 mg/Kg, PCP plasma concentration data was best fit with a two compartment model in male rats and a one compartment model in female rats. The calculated bioavailability of PCP after oral gavage doses ranged from 47 to 75%, which appeared to be related to incomplete GI tract absorption. (Both sexes had a similar rate of GI tract absorption of PCP but the area under concentration versus time curves (AUC) values obtained from both the iv and gavage routes were significantly different with males having higher AUC values.) The theoretical PCP plasma concentrations in a proposed chronic dosed feed study were simulated using a computer model. Results of the simulation suggest that if no significant palatability problems exist, PCP concentrations in rat plasma will fluctuate daily and a quasi-steady state will be achieved after <i>ad libitum</i> exposure to dosed feed for approximately 4 days.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21169-01 CCB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Toxicokinetics of Pentachloroanisole in F344 Rats and B6C3F1 Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	J.H. Yuan	Visiting Associate	CCB	NIEHS
Others:	T.J. Goehl	Ph.D.	CCB	NIEHS
	R. Irwin	Ph.D.	CCB	NIEHS
	H.L. Hong	Biologist	CCB	NIEHS

COOPERATING UNITS (if any)

Comparative Medicine Branch

LAB/BRANCH

Chemical Carcinogenesis Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The toxicokinetics of pentachloroanisole (PCA) was studies in F344 rats and B6C3F1 mice of both sexes at doses of 10, 20 and 40 mg/Kg. A rapid elimination of PCA and a rapid formation of pentachlorophenol (PCP) was observed in both species after an iv or an oral dose of PCA. No sex differences were found in the rate of absorption of PCA from the GI tract or in the overall rate of elimination of PCA. The estimated area under concentration versus time curves (AUC) of PCA after gavage or iv administration is similar for both sexes and both species. However, the AUC of the metabolite (PCP) was significantly larger in female rats than in male rats at all gavage doses. No difference was observed in mice. The AUC of PCA increased disproportionately with dose in both species. The results of these studies indicate that the observed sex differences of rats and mice in toxic and carcinogenic response to PCA is not related to the systemic availability of PCA or to the rate of demethylation of PCA to PCP. The high plasma concentrations and relatively long biological half-life of PCP in both species after iv and oral dosing with PCA indicates that bioaccumulation of PCP will occur upon multiple oral administration of PCA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21170-01 CCB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Toxicokinetics of Oxazepam in F344 Rats, B6C3F1 Mice and Swiss Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	J.H. Yuan	Visiting Associate	CCB	NIEHS
Others:	T.J. Goehl	Ph.D.	CCB	NIEHS
	R. Irwin	Ph.D.	CCB	NIEHS
	H.L. Hong	Biologist	CCB	NIEHS

COOPERATING UNITS (if any)

Comparative Medicine Branch

LAB/BRANCH

Chemical Carcinogenesis Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The toxicokinetics of oxazepam were studied in F344 rats, B6C3F1 mice and Swiss mice. Elimination of oxazepam appears to be a first order process in all species. A sex difference in distribution and elimination was observed in F344 rats. At the same dosage (oral or iv), oxazepam plasma concentrations in male rats are significantly lower than in female rats. In rats, oxazepam plasma concentrations tend to increase when the oral gavage dose is increased from 50 to 200 mg/Kg. However, there was no further significant increase of plasma concentrations at 400 mg/Kg dosage, which is believed to be caused by incomplete absorption. Bioavailability of oxazepam from the gavage route is poor. The calculated bioavailability based on dose normalized area under concentration versus time curves (AUC) values ranged from 26 to 43%. There was no obvious sex differences in bioavailability.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21172-01 CCB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Modeling Internal Doses in Rodent Feed and Drinking Water Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: J.H. Yuan Visiting Associate CCB NIEHS

Others:

COOPERATING UNITS (if any)

LAB/BRANCH

Chemical Carcinogenesis Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A computer model for estimating internal dose in dosed feed and dosed drinking water studies has been developed. The model was constructed based on linear theory. The animal's feeding and drinking habits, as well as the linear kinetics of the test chemical in animals were built into the model. Blood concentrations of test chemicals in dosed feed and in dosed drinking water studies were simulated using the developed model. The actual blood concentrations in rodents administered test chemical in feed and drinking water studies were compared to those predicted by the model. Results of the modeling studies accurately predicted the blood concentrations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21176-01 CCB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Toxicokinetics of Emodin in F344 Rats, and B6C3F1 Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	J.H. Yuan	Visiting Associate	CCB	NIEHS
Others:	T.J. Goehl	Ph.D.	CCB	NIEHS
	R.Irwin	Ph.D.	CCB	NIEHS
	H.L. Hong	Biologist	CCB	NIEHS

COOPERATING UNITS (if any)

Comparative Medicine Branch

LAB/BRANCH

Chemical Carcinogenesis Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Toxicokinetics of emodin are being studied in F344 rats and B6C3F1 mice. Bioanalytical method for quantitation of emodin and its glucuronide conjugate have been developed. Groups of rats and mice have been dosed with emodin intravenously at one dose or orally at three doses. Plasma concentrations of emodin and its glucuronide conjugate are being determined. Preliminary data indicate a relatively rapid elimination of emodin. Bioavailability of emodin will be estimated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21177-01 CCB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Toxicokinetics of Chlorpyrifos in F344 Rats, and B6C3F1 Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	J.H. Yuan	Visiting Associate	CCB	NIEHS
Others:	T.J. Goehl	Ph.D.	CCB	NIEHS
	K. Abdo	Ph.D.	CCB	NIEHS
	H.L. Hong	Biologist	CCB	NIEHS

COOPERATING UNITS (if any)

Comparative Medicine Branch

LAB/BRANCH

Chemical Carcinogenesis Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Toxicokinetics of chlorpyrifos are being studied in F344 rats and B6C3F1 mice. Bioanalytical method for quantitation of chlorpyrifos and its main metabolite are being developed. Plasma concentrations of chlorpyrifos and its main metabolite will be determined after bolus iv injection of chlorpyrifos and gavage administration of chlorpyrifos. Bioavailability of chlorpyrifos will be estimated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201-ES-21178-01 CCB

PERIOD COVERED

October 1, 1991 - September 30, 1992 TERMINATED

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pancreas Tumor Transplantation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	H.L. Hong	Biologist	CCB	NIEHS
Others:	G.A. Boorman	D.V.M., Ph.D., Chief	CCB	NIEHS
	R. Irwin	Ph.D.	STB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Chemical Carcinogenesis Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The increasing incidence of human exocrine pancreatic cancer has created a need for an adequate animal model for human disease. The objective of this study is to determine whether DSL-6 transplantable acinar cell tumor will undergo phenotype shifts when grown in culture and then transplanted back into rats. The basic goal is to regraft the tumor and see what the phenotype is after a period in culture.

A transplantable tumor as DSL-6 was derived from a primary acinar cell carcinoma of the pancreas. The carcinoma developed in a male Lewis rat that was given 3 intraperitoneal 30 mg/Kg injections of azaserine (a diazoketone carcinogen) when the rat was 3 weeks of age. Histologically, the tumor was a moderately well differentiated acinar cell carcinoma with a microcystic phenotype. Serial subcutaneous transplants have grown as moderately to poorly differentiated acinar cell carcinomas. These transplants served as the source of cells for culture. After 4-6 weeks, cultured tumor cells were inoculated subcutaneously into young male Lewis rats. Tumors from rats were collected and examined by light microscopy and electron microscopy. The rat model has been quite useful to understand tumor histogenesis, even though the pancreatic neoplasms they develop do not resemble those that constitute the most common type encountered in humans, namely ductal adenocarcinomas.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21051-09 ECMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TERMINATED September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cytogenetic Analysis of Mutagen-Sensitive Mutants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: James M. Mason Geneticist ECMB NIEHS
Larry E. Champion Biologist ECMB NIEHS

COOPERATING UNITS (if any)

University of California, Davis

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

SECTION

Heritable Effects Research Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21053-09 ECMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Control of Mutation in *Drosophila*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

James M. Mason, Geneticist, ECMB, NIEHS
 Min Wang, Visiting Fellow, ECMB, NIEHS
 Larry E. Champion, Biologist, ECMB, NIEHS

COOPERATING UNITS (if any)

University of California, Irvine
 Massachusetts Institute of Technology

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

SECTION

Heritable Effects Research Group

INSTITUTE AND LOCATION

National Institute of Environmental Health Sciences, Research Triangle Park, NC

TOTAL STAFF YEARS:

2.9

PROFESSIONAL:

1.9

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is designed to determine the relationship between DNA repair, chromosome structure and mutagenesis in *Drosophila melanogaster*. A mutation that increases the mutation frequency (a mutator) has been identified and characterized. The mutator greatly reduces the efficacy of repair of x-ray induced chromosome breaks, thereby allowing a previously undescribed repair pathway to be observed. By this newly identified repair pathway broken chromosome ends are "capped" with a new telomere. The new chromosome ends are protected from degradation. These broken ends are not replicated effectively and DNA sequences are lost from the capped ends. Quasi-stable derivatives of the capped ends have been isolated and found to have gained a telomere-specific DNA sequence which has a structure resembling a retrotransposon. Preliminary evidence suggests that natural chromosome ends also recede because of incomplete DNA replication and occasionally lengthen by the acquisition of this telomere-specific sequence. The rate of loss is balanced by the rate of transposition to maintain chromosome length. We are also developing a rapid assay for the mutator to facilitate genetic analysis and investigating cell specificity of mutator activity. The mutator gene maps to polytene region 62B12-C1. Yeast artificial chromosomes that cover the region are being subcloned to identify a transcription unit that corresponds to the mutator gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21094-06 ECMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mutagenesis and Other Cellular Responses to Chemicals that Generate Free Radicals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

Errol Zeiger, Supervisory Microbiologist, ECMB/NIEHS

COOPERATING UNITS (if any)

A.-A. Stark, University of Tel-Aviv, Israel

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Group

SECTION

Mutagenesis Group

INSTITUTE AND LOCATION

National Institute of Environmental Health Sciences, Research Triangle Park, NC

TOTAL STAFF YEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The formation of hydrogen peroxide (H_2O_2) and free radicals from glutathione (GSH) is dependent on the activity of g-glutamyltranspeptidase (GGT), an enzyme that is frequently present at high levels in preneoplastic cells. GSH, in the presence of GGT, can induce lipid peroxidation in vitro, using linolenic acid or linoleic acid, or microsomal membranes as substrates, and in situ in cultured human hepatoma cells. Mutagenicity of Salmonella and lipid peroxidation occurs in the presence of physiological concentrations of GSH, iron and copper, and endogenous chelators, such as citrate, ADP, transferrin, and ceruloplasmin. Unlike the mutagenic effect of GSH which is mediated through the formation of H_2O_2 , lipid peroxidation appears to be effected through a free radical reaction, without the intermediate formation of H_2O_2 .

The effects of antioxidants and free radical scavengers on mutagenicity and lipid peroxidation induced by other sulfhydryls, such as D- and L-cysteine has being studied. The results suggest that these thiols are mutagenic via the same mechanisms as GSH, i.e., through the formation of H_2O_2 .

Preliminary studies have shown that lipid peroxidation can be induced in GGT-rich preneoplastic foci in rodents when challenged with GSH. Histochemical studies show that areas of lipid peroxidation are contiguous with GGT-rich preneoplastic foci in the livers of rats treated with the carcinogen diethylnitrosamine, and a promoter of liver carcinogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21103-06 ECMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluations of Genetic Toxicity Test Results

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

Errol Zeiger, Supervisory Microbiologist, ECMB/NIEHS
Beth Anderson, Biologist, ECMB/NIEHS
Walter Piegorsch, Mathematical Statistician, SBB/NIEHS

COOPERATING UNITS (if any)

Barry Margolin, Department of Biostatistics, UNC, Chapel Hill, NC

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

SECTION

Mutagenesis Group

INSTITUTE AND LOCATION

National Institute of Environmental Health Sciences, Research Triangle Park, NC

TOTAL STAFF YEARS:

0.4

PROFESSIONAL:

0.4

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A database of NTP short-term genetic toxicity test results and the results from carcinogenesis tests has been created. This database allows the evaluation of each short-term assay with respect to its ability to predict carcinogenesis or other short-term assay results. It also permits studies of the individual assays with respect to inter- and intra-laboratory reproducibility. Examination of test predictivity as a function of the potency of the short-term test (STT) and carcinogenicity test is continuing. The chemicals being evaluated comprise the 114-chemical dataset used previously to correlate qualitative STT results with carcinogenicity results. The short-term genetic toxicity results are being integrated with other toxicity and biochemical parameters of these chemicals in an attempt to improve the prediction of carcinogenicity and other toxic effects.

A number of potency measures have been proposed for the Salmonella and carcinogenesis tests, but there are no commonly-accepted measures of potency for the other STTs, such as in vitro chromosome aberrations, in vitro SCE, or the mouse lymphoma test. The analyses of the potency measures for Salmonella has been completed. The measurements used for Salmonella are being redefined to fit the data from the mammalian cell assays, and will be used to evaluate the relationships of the in vivo tests with the carcinogenic potencies for the same chemicals.

The database of Salmonella results was used to evaluate the ability of a number of procedures to predict mutagenicity. The predictivities for Salmonella mutagenicity of two computer-based structure-activity (SAR) systems, one empirical structure-activity system, and one physicochemical system, were determined for 100 chemicals. The chemical structures, or coded chemicals, were sent, without identifying them by name, to the participants. The study was designed to: measure the accuracy of the different test systems; identify the strengths and weaknesses of each system; and bring greater understanding to structure-activity predictions. The two computer-based SAR systems and empirical system gave equivalent predictivities (approx. 70-80%), while the physicochemical system was less effective (approx. 60%).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21121-04 ECMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Transfection of cDNAs for Drug Metabolizing Enzymes into Mammalian Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

Robert Langenbach, Microbiologist, ECMB/NIEHS
Howard Tiano, Biologist, ECMB/NIEHS
Masakiyo, Hosokawa, Visiting Associate, ECMB/NIEHS
Pat Chulada, Graduate Student, ECMB/NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

SECTION

Metabolism and Molecular Mechanisms Group

INSTITUTE AND LOCATION

National Institute of Environmental Health Sciences, Research Triangle Park, NC

TOTAL STAFF YEARS:

2.1

PROFESSIONAL:

2.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To develop more efficient in vitro systems for the study of pro-mutagenic or pro-carcinogenic chemicals, we have developed transgenic C3H/10T $\frac{1}{2}$ cell lines expressing human cytochrome P450 (CYP) 2A6. A retroviral vector containing the cloned CYP2A6 cDNA was packaged in psi-2 cells, and used to infect C3H/10T $\frac{1}{2}$ cells. From approximately 100 G418 resistant clones, three cell lines were chosen for further study based upon their morphologies, growth rates, and CYP2A6-dependent coumarin 7-hydroxylase activities. The 10T $\frac{1}{2}$ cells and infected clone 10T $\frac{1}{2}$ -04 had no detectable CYP2A6 enzyme activity, while clones 10T $\frac{1}{2}$ -10 and 10T $\frac{1}{2}$ -19 had microsomal CYP2A6 activities within the range of those found in human liver microsomes. Western analysis was in agreement with the observed enzyme activities. By Southern analysis, clone 10T $\frac{1}{2}$ -04 had one vector copy per cell while clones 10T $\frac{1}{2}$ -10 and 10T $\frac{1}{2}$ -29 had 6 and 5 copies per cell, respectively. Southern analysis also indicated the presence of endogenous CYP2A6 hybridizable DNA in the four cell lines. All exhibited about equal sensitivity to the cytotoxicity of, and induction of ouabain resistance by the direct acting mutagen N-methyl-N'-nitro-N-nitrosoguanidine. The four lines were about equally sensitive to transformation by benzo(a)pyrene, a chemical requiring metabolic activation. However, only clones 10T $\frac{1}{2}$ -10 and 10T $\frac{1}{2}$ -29, which express CYP2A6 activity, were able to be mutated and morphologically transformed by the cigarette smoke carcinogen, 4-(methylnitroso)-1-(3-pyridyl)-1-butanone, NNK. In addition, studies performed on a subset of the original 100 clones to relate enzyme activity to copy number showed that the copy number ranged from one to eight, and that enzyme activity varied linearly with the number of copies integrated. These data, coupled with the ease of the enzyme assay, indicate that CYP2A6 should make a good reporter gene in transgenic systems. ASS2 cells expressing CYP2A6 have been derived. These cells have been treated with NNK and, in collaboration with Dr. Kenneth Tindall, the mutagenic spectrum in the resultant mutated clones is being studied. These studies should improve the usefulness of cultured mammalian cells for the identification and investigation of the biological effects of environmental chemicals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21133-03 ECMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TERMINATED September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Comparative Genetic Toxicology of H.C. Blue 1 and H.C. Blue 2

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

P.I.: R. Langenbach Microbiologist ECMB NIEHS

Others: F. Kari Biologist STB NIEHS
A. Abu-Shakra Visiting Fellow ETB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

SECTION

Metabolism & Molecular Mechanisms Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

.5

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21157-02 ECMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Analysis of Cell Cycle Control During Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

Richard S. Paules, Toxicologist, ECMB/NIEHS

Cynthia L. Innes, Biologist, ECMB/NIEHS

C. Nelson Rhodes, II, Postdoctoral IRTA, ECMB/NIEHS

COOPERATING UNITS (if any)

Molecular Oncology Section, NCI-FCRDC

Department of Pathology, UNC-CH School of Medicine

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

SECTION

Mammalian Molecular Genetics Group

INSTITUTE AND LOCATION

National Institute of Environmental Health Sciences, Research Triangle Park, NC

TOTAL STAFF YEARS:

2.4

PROFESSIONAL:

1.4

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to gain insight into alterations of cell cycle control mechanisms in neoplastically transformed mammalian cells. A requisite step in the process of carcinogenesis is the loss of normal control of cellular proliferation. This can result, at least in part, from gain-of-function mutations (such as activations of oncogenes), loss-of-function mutations (such as inactivations of normal tumor suppressor gene functions), and both heritable and non-heritable alterations in the normal pattern of gene expression and function. We are studying alterations in growth control signal transduction pathways in genetically altered mammalian cells in culture as compared with their normal counterparts. Specifically, we are investigating cell cycle related parameters in mouse fibroblasts that constitutively overexpress specific oncogene products and in primary embryo cultures from mice with one or both alleles of the p53 tumor suppressor gene inactivated. We have been carefully defining the cell division cycle kinetics of clonal populations of NIH3T3 cells or NIH3T3 cells overexpressing *mos^{mu}*, *mos^{ke}*, *H-ras^{val12}*, *trp-met*, *v-src*, and *c-src^{mu}*. These studies involve optimizing synchrony protocols through the use of various combinations of either serum deprivation, mitotic shake-off, hydroxyurea treatment, or aphidicolin treatment. The degree of synchrony is being monitored by such parameters as incorporation of ³H-thymidine or BrdU into newly synthesized DNA, mitotic indices, and population doublings. We are investigating cell cycle related parameters such as the level of expression and phosphorylation status of the *cdc2*-family of kinases, cyclin proteins, tubulin proteins, reorganization of interphase microtubules to mitotic spindles, and phosphorylation events associated with the activation of microtubule organizing centers (MTOCs) and maturation promoting factor (MPF) activity. Additional studies will follow specific gene products (such as *p34^{cdc2}*, cyclin proteins, and p53) in genetically altered cells as compared to their normal counterparts as they progress through their division cycle following exposure to environmental genotoxic and non-genotoxic carcinogens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21158-02 ECMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oncogenic Activity of Retroviral Vectors Used in Human Gene Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

Robert Langenbach, Microbiologist, ECMB/NIEHS
 Ren-Li Wang, Visiting Scientist, ECMB/NIEHS
 Howard Tiano, Biologist, ECMB/NIEHS

COOPERATING UNITS (if any)

Dr. French Anderson, NIH

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

SECTION

Metabolism and Molecular Mechanisms Group

INSTITUTE AND LOCATION

National Institute of Environmental Health Sciences, Research Triangle Park, NC

TOTAL STAFF YEARS:

1.9

PROFESSIONAL:

1.9

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The first federally approved clinical trial of retroviral vector based gene therapy for human genetic disease has begun. There are several concerns that must be addressed when applying retroviral-mediated gene transfer to human somatic cell genetic diseases. One concern is that the vector, or components of it, may have oncogenic effects. We are investigating whether the use of retroviral vectors for gene therapy may have adverse consequences by examining the effects of such vectors on transformation of cells in vitro. The initial phase of the project involved infecting C3H10T½ cells with the retroviral vector backbone PE501/G1Na (LTR-neo-LTR), ecotopically packaged and supplied by Dr. Anderson (NIH). This vector was found to efficiently infect C3H10T½ cells and to confer resistance to the antibiotic G418, to transfected cells. Transformation experiments were performed under three different regimens: a single exposure to the vector at varying titer, repeated exposures, and exposure followed by replating of the cells. Southern blots show that the vector integrated into the DNA of the infected cells and that the number of integrated vector copies increased with increasing titer of the vector. Each experiment measured three effects: cytotoxicity, G418 resistance, and transformation. 3-methylcholanthrene (3-MC), a known carcinogen which is transforming but relatively nontoxic, served as a positive control. In no case did exposure to the vector, PE501/G1Na, cause any decrease in survival or increase in transformation of C3H10T½ cells compared to untreated (negative) controls.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21160-02 ECMB

PERIOD COVERED

December 1, 1990 through September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mutagenicity Studies in Cultures Mammalian Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

Songyun Zhu, Visiting Fellow, ECMB/NIEHS

Errol Zeiger, Supervisory Microbiologist, ECMB/NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

SECTION

Mutagenesis Group

INSTITUTE AND LOCATION

National Institute of Environmental Health Sciences, Research Triangle Park, NC

TOTAL STAFF YEARS:

1.1

PROFESSIONAL:

1.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cytotoxicity and mutagenicity of the human carcinogen treosulphan, and its hydrolysis product, dl-1,2:3,4-diepoxybutane (DEB), were studied in AS52 cells, a PSV2gpt-transformed Chinese hamster ovary cell line. Factors such as the pH of the treatment medium, the duration of exposure, and the cell density during the treatments, were also investigated. Both chemicals were toxic and mutagenic. The toxicity and mutagenicity of treosulphan was pH-related, whereas the DEB effects were pH-independent. This finding supports the hypothesis that (pH-dependent) hydrolysis of treosulphan is necessary for the mutagenicity of this chemical.

The molecular nature of the treosulphan and DEB-induced mutants are being investigated to ascertain whether both chemicals were mutagenic via the same ultimate mutagen. PCR analyses of restriction fragments from treosulphan and DEB-induced mutants showed similar patterns; 23% and 24% of the mutants carried point mutations, 26% and 21% had partial deletions of the targeted chromosome region, and 52% and 55% had complete deletions of this region, for treosulphan and DEB, respectively. These results suggest that the same molecular moiety is responsible for the mutagenicity of both chemicals. Studies are in progress to sequence the point mutations.

Although metal salts are usually reported as nonmutagenic in cultured mammalian cells, preliminary studies have shown that a number of these salts are weakly mutagenic in the AS52 cells under the treatment conditions used in our laboratory. These mutagenic responses, and the effects of protocol variations on their presence and magnitude, are being further characterized. The mutants obtained will be sequenced to determine the precise modes of action of the different metals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21161-02 ECMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Predictive Toxicology Methods

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

Douglas W. Bristol, Chemist, ECMB/NIEHS
Raymond W. Tennant, Supervisory Biologist, ECMB/NIEHS
Dennis R. Bahler, Computer Scientist, ECMB/NIEHS
Michael R. Elwell, Veterinary Pathologist, ECMB/NIEHS

COOPERATING UNITS (if any)

Department of Chemistry, Case Western Reserve University, Cleveland, OH
Department of Computer Science, North Carolina State University, Raleigh, NC

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

SECTION

Cancer Biology Group

INSTITUTE AND LOCATION

National Institute of Environmental Health Sciences, Research Triangle Park, NC

TOTAL STAFF YEARS:

1.2

PROFESSIONAL:

1.0

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The development of methods that predict the toxicity expected for any untested chemical of interest, based on knowledge of the chemical's structure, substructure, physicochemical behavior, or biological behavior in short-term or in vitro tests, can lead to a better understanding of mechanisms that underlie toxic modes of action and of factors that influence inter-species extrapolation. They can also greatly enhance management of the selection of chemicals for testing and the identification of tests that most need to be conducted, thereby reducing the cost of testing programs and lessen their dependence on animals. Past efforts to develop predictive methods based on NTP standardized tests for mutagenicity (four in vivo short-term test), Balb/c-3T3 mouse embryo cell cytotoxicity and transformation, and carcinogenicity indicate that systematic analyses of organ-specific, histopathology data from NTP subchronic and chronic toxicity studies is a most promising direction in which to extend this work. The abstraction and compilation of such organ-specific histopathology data from NTP Technical Reports for the 200 most-recently studies chemicals is in progress and will be completed during the next year. The organ-specific, histopathology data, along with other parameters, will be analyzed using new computer-based approaches developed in the field of artificial intelligence that are especially capable of identifying patterns and correlations embedded in large amounts of seemingly unrelated data. This interdisciplinary work will be accomplished through collaborations with Dr. G. Klopman at Case Western Reserve University, using a particularly promising knowledge-based computer system call CASE (computer assisted substructure evaluation) and with Dr. D. Bahler at North Carolina State University, using artificial intelligence approaches. Collaborations will also be developed through the DERT's Small Business Innovative Research (SBIR) program.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-21162-02 ECMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Environmental Chemical and Oxidative Stress Induced DNA Damage and Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

John E. French, Physiologist, ECMB/NIEHS

Amy C. Johnson, Biology Technician, ECMB/NIEHS

Kenneth Tindall, Microbiologist, ECMB/NIEHS

Raymond W. Tennant, Supervisory Biologist, ECMB/NIEHS

COOPERATING UNITS (if any)

Ray Tice, Ph.D., Integrated Laboratory Systems, RTP, NC

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

SECTION

Cancer Biology Group and Heritable Effects Research Group

INSTITUTE AND LOCATION

National Institute of Environmental Health Sciences, Research Triangle Park, NC

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

0.6

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Endogenous oxidative injury to cellular DNA may be induced by exogenous chemical toxicity and/or inflammation and has been speculated to contribute to mutation frequency (MF) and mutations that may be fixed and clonally expanded (multistage carcinogenesis) by induced cellular proliferation. Our goal is to determine the mutation frequency and to characterize mutations in transgenic ZAP/lacI^q mice (C57Bl/6 background) using a lambda shuttle vector after treatment with selected genotoxic or non-genotoxic chemicals that induce hyperplasia with and without associated inflammation as a model for genomic DNA damage. The target transgene is recovered from treated transgenic mice by exposing the mouse genomic DNA to λ in vitro packaging extracts, infecting repair deficient host E. coli, and culturing. Phage with mutations in the lacI target gene form colored plaques, while those with non-mutated target genes form colorless plaques. We have initiated these studies by determining mutation frequency in liver after ethylnitrosourea or vehicle exposure and induction of rapid hepatocellular proliferation following 1/4 partial hepatectomy. Ablated liver served as the T₀ and untreated control. After 8 days (100% restoration of liver mass) EtNU followed by hepatectomy increased MF 4.4X compared to $\approx$ 1.9 or 1.7X for hepatectomy alone or EtNU and sham hepatectomy. Additional studies performed with benzene, p-cresidine, 1,2,3-trichloropropane and 4-vinyl-1-cyclohexene diepoxide are undergoing analysis. Using this research strategy we expect to be able to provide insight on chemical and/or endogenous oxidative damage to DNA and this possible mechanism of carcinogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21173-01 ECMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Karyotypic Analysis of Malignant Skin Tumors of TG α AC (v-Ha-ras) and FVB Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

John E. French, Physiologist, ECMB/NIEHS
Judson Spalding, Biologist, ECMB/NIEHS
Raymond W. Tennant, Supervisory Biologist, ECMB/NIEHS
Laura Hansen, Biologist, ECMB/NIEHS

COOPERATING UNITS (if any)

Ray Tice, Ph.D., Integrated Laboratory Systems

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

SECTION

Cancer Biology Group

INSTITUTE AND LOCATION

National Institute of Environmental Health Sciences, Research Triangle Park, NC

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Insertion of v-Ha-ras (TG α AC mice; ζ -globin promoted v-Ha-ras) into FVB mice eliminates the initiation step in carcinogen initiated and promoted mouse skin tumorigenesis. Along with point mutations in both v-Ha-ras and c-Ha-ras, trisomies of chromosomes 6 and 7, c-Ha-ras allelic imbalance, and LOH on chromosome 7 have been reported to be associated with tumor progression from benign papillomas to squamous cell carcinomas or spindle cell tumors. Sequential topical application of tumor promoting agents to TG α AC (ζ -globin promoted v-Ha-ras initiated) transgenic mice resulted in papilloma induction followed by the appearance of disc-shaped soft tissue masses with an ulcerating surface. Karyotypic analysis of these tumors indicated a highly consistent pattern of trisomy of chromosomes 10 and 6 and monosomy of chromosome 13. The spindle shaped cells from primary cultures were highly tumorigenic when transplanted to 9 to 11 day old syngeneic recipients, resulting in large disc-shaped or larger oval shaped ulcerating tumors within approximately two weeks. Western analysis of these transplanted tumors indicate that the transgene (v-Ha-ras) is highly expressed compared to normal skin from transgenic animals and induced papillomas. These results indicate that these spindle shaped cell tumors initiated by insertion of this v-Ha-ras transgene followed by chemical promotion involve complex chromosome arrangements which may be important in identifying other oncogenes and/or tumor suppressor genes involved in skin tumorigenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21174-01 ECMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Transgenic Mouse Model for Dermal Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

Judson W. Spalding, Biologist, ECMB/NIEHS
John French, Physiologist, ECMB/NIEHS
Laura Hansen, Biologist, ECMB/NIEHS
Carl Szczesniak, Stay-in-Schooler, ECMB/NIEHS
Thai-Vu Ton, Biologist, ECMB/NIEHS

COOPERATING UNITS (if any)

Integrated Laboratory Systems, NC
Environmental Health Research and Testing, NC

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Group

SECTION

Cancer Biology Group

INSTITUTE AND LOCATION

National Institute of Environmental Health Sciences, Research Triangle Park, NC

TOTAL STAFF YEARS:

1.75

PROFESSIONAL:

1.25

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this project is to develop the TG.AC transgenic mouse line as a model for studying the multistage process of skin carcinogenesis. The TG.AC transgenic mouse line carries a v-Ha-ras oncogene driven by a zeta-globin promoter which expresses in the skin and confers on the skin the properties of a genetically initiated tissue. The model offers the opportunity to (1) identify chemicals that are promoters or complete carcinogens in the skin; (2) study the molecular mechanisms associated with the induction of papillomas and progression to malignancy; (3) study mechanisms of chemically induced expression or repression of the transgene; and (4) identify the regulatory mechanisms that determine the specificity for transgene expression in the skin. We have demonstrated that the TG.AC mouse line is very sensitive to such known promoters as TPA, benzoyl peroxide, and methylethyl ketone peroxide. Wounding also readily induces papillomas. Our results indicate that expression of the transgene is confined to the skin; for urethan, a potent systemic rodent lung carcinogen, induced only lung tumors in TG.AC mice and at the same incidence as in wildtype nontransgenic FVB mice. Under optimal conditions, a single application of TPA is sufficient to induce one or several papillomas in 10% of treated TG.AC mice. The age of the mice may be an important factor contributing to the relative sensitivity of TG.AC mice to tumor induction. A higher papilloma incidence and shorter latency period appear to be associated with promoter treatment in older mouse groups. Primary keratinocyte cultures from TG.AC mice are being developed in order to study growth regulatory factors that can modify expression of the transgene or for studying chemically induced transformation in vitro. Other studies are being conducted to determine whether the TG.AC model can serve as a short term in vivo model for identifying chemical promoters and/or complete carcinogens that may be hazardous to human health.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01ES 21175-01 ECMB

PERIOD COVERED

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

v-Ha-ras expression, proliferation and skin tumorigenesis in ras transgenic mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

Laura A. Hansen, Biologist, ECMB/NIEHS

Raymond W. Tennant, Supervisory Biologist, ECMB/NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

SECTION

Cancer Biology Group

INSTITUTE AND LOCATION

National Institute of Environmental Health Sciences, Research Triangle Park, NC

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Activation of the ras proto-oncogene has been found in multiple human malignancies and is believed important in many tumorigenesis pathways. We are using a transgenic mouse model which has a structural v-Ha-ras transgene fused to a fetal zeta-globin promoter to study the events of tumorigenesis associated with ras activation. These TG-AC strain transgenic mice rapidly develop large numbers of epidermal papillomas following TPA treatment, some of which progress to malignancy. Histologically, the skin of the TG-AC mice is undistinguishable from that of noncarrier FVB mice and responds with a similar down-regulation of protein kinase C and hyperplasia following tetradecanoylphorbol-13-acetate (TPA) treatment, except for the focal appearance of papillomas on TG-AC skin. Using the techniques of Western blotting and in situ hybridization histochemistry we have localized and quantitated transgenic ras mRNA and protein product expression in epidermal papillomas and are in the process of examining transgene expression in malignancies of the skin and other tissues. Both Western blotting and in situ hybridization histochemistry have demonstrated that the transgene message and protein product are expressed in epidermal papillomas but not in normal or TPA-treated skin. Using in situ hybridization histochemistry experiments the transgene message was further localized to the basal epidermoid cells of the papillomas. Immunohistochemical analysis for PCNA (proliferating cell nuclear antigen) which is a marker for dividing cells localizes proliferating cells of the papilloma to this same basal epidermoid cell population. Thus, using in situ hybridization histochemistry for the transgene message together with immunohistochemical analysis of dividing cells we have shown an association of transgenic ras expression and proliferation in the papillomas. Other experiments with papilloma precursors have suggested that the papillomas develop focally from keratinocytes associated with the hair follicle. Future experiments will examine the role of the hair follicle in papilloma formation and pursue the association of activated ras expression and proliferation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-60102-14 ECMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Testing of Chemicals of Interest in Salmonella

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

Errol Zeiger, Supervisory Microbiologist, ECMB/NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

SECTION

Mutagenesis Group

INSTITUTE AND LOCATION

National Institute of Environmental Health Sciences, Research Triangle Park, NC

TOTAL STAFF YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies on the mutagenicity of divalent metals has shown that in addition to cobalt chloride (Co^{2+}), ferrous sulfate (Fe^{2+}), cadmium chloride (Cd^{2+}), and zinc chloride (Zn^{2+}), manganous sulfate (Mn^{2+}), and nickel sulfate (Ni^{2+}), are mutagenic in *Salmonella typhimurium*. Mutagenicity was detected only when preincubation was done in distilled, deionized water or HEPES buffer. Phosphate buffer or components of Vogel-Bonner medium, used in the standard mutagenicity studies, inhibited the mutagenicity of these metals. Studies of the specificity of mutagenesis in the different *Salmonella* strains have shown that metals can be detected as mutagens in both base-pair substitution and frameshift strains of *Salmonella*, and that the plasmid, pKM101, is required for mutagenesis. Under these same conditions, there was no evidence of mutagenicity for any of the lead (Pb^{2+}) salts tested, and copper (Cu^{2+}) salts produced equivocal responses.

These data show that the traditional inability to detect these metals as mutagens in *Salmonella* is not due to an inherent insensitivity of the *Salmonella*, but to the interaction of the metals with media components and/or passive and active transport processes.

Terminated 12/91.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-65050-06 ECMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Analysis of Deletion Mutations in Chinese Hamster Ovary Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

Kenneth R. Tindall, Geneticist, ECMB/NIEHS
Zsolt Kelecsenyi, Visiting Fellow, ECMB/NIEHS

COOPERATING UNITS (if any)

Dr. William D. Caspary, ETB, DTRT, NIEHS, RTP, NC
Dr. Sid Aaron, The Upjohn Co., Kalamazoo, MI
Dr. Leon F. Stankowski, Jr., Pharmakon Research International, Inc., Waverly, PA

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Group

SECTION

Heritable Effects Research Group

INSTITUTE AND LOCATION

National Institute of Environmental Health Sciences, Research Triangle Park, NC

TOTAL STAFF YEARS:

0.9

PROFESSIONAL:

0.9

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Genomic rearrangements are among the most serious mutations arising in mammalian cells, implicated in the process of carcinogenesis and in the etiology of a variety of human genetic disorders. While many mutagens and carcinogens induce genomic rearrangements, most mammalian mutagenesis assays are limited in their ability to detect large scale genomic mutations. Often genomic rearrangements are lethal to the cell, however, the mechanisms by which such rearrangements result in viable cells deserve further study. To study the mechanisms by which genomic rearrangements arise, we use the transgenic Chinese hamster ovary cell line, AS52, which carries a single functional copy of the bacterial gpt gene stably integrated into the CHO genome. AS52 cells are well defined as being capable of detecting mutations induced by a variety of clastogens and radio-mimetic agents that are not detected as mutagens in other mammalian mutational assay systems, apparently due to the location of the genomic integration of the bacterial gpt transgene. We are characterizing the genomic site of gpt integration and have defined a nonfunctional gpt/plasmid related sequence that is closely linked to the functional gpt gene. In order to rapidly distinguish deletions and rearrangements from putative point mutations, we have developed primers and reaction conditions useful for multiplex PCR of AS52 genomic DNA. Our multiplex PCR yields sequences from the dhfr gene (which is not linked to gpt) as well as from both the functional and the nonfunctional gpt sequences. The nonfunctional gpt sequence can serve as a marker for rapidly distinguishing smaller deletions/rearrangements while the dhfr sequences serve as an internal PCR control. If the PCR conditions are appropriate then we should always observe the dhfr PCR fragment, while loss of both the functional and the nonfunctional gpt related PCR fragments allows the distinction of larger scale deletion/rearrangement events. Thus, we can now rapidly distinguish complete deletions (i.e., large scale genomic rearrangements) from partial deletions (i.e., smaller scale genomic rearrangements), from point mutations simply using PCR. We are presently characterizing the molecular fine structure of deletions induced by the base analog, 5-azacytidine, and the chemotherapeutic agent, U73,975.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-65051-06 ECMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Analysis of Point Mutations in Chinese Hamster Ovary Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

Kenneth R. Tindall, Geneticist, ECMB/NIEHS
Zsolt Kelecsenyi, Visiting Fellow, ECMB/NIEHS
Jessica Sneed, Stay-in-School, ECMB/NIEHS

COOPERATING UNITS (if any)

Dr. Leon F. Stankowski, Jr., Pharmakon Research International, Inc., Waverly, PA
Dr. Sid Aaron, The Upjohn Co., Kalamazoo, MI
Dr. William Caspary, ETB, and Drs. E. Zeiger and S-Y Zhu, ECMB/NIEHS

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

SECTION

Heritable Effects Research Group

INSTITUTE AND LOCATION

National Institute of Environmental Health Sciences, Research Triangle Park, NC

TOTAL STAFF YEARS:

1.6

PROFESSIONAL:

1.1

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We use the Chinese hamster ovary (CHO) cell line (AS52) with a single functional bacterial gpt transgene stably integrated into the genome to study point mutational changes in mammalian cells. Mutants arise at the gpt locus as 6-thioguanine resistant (6Tgr) colonies and are characterized as carrying putative point mutations or deletion mutations using the polymerase chain reaction (PCR). Point mutational spectra are then generated using DNA sequence analysis. We have recently implemented methods in the laboratory to simplify the molecular analysis of mutations in mammalian cells. The site of a mutation in a PCR fragment can be quickly identified prior to DNA sequencing using the HOT (hydroxylamine or osmium tetroxide) modification protocol. Such an approach limits the amount of DNA sequencing necessary and allows for the sequence characterization of a substantially greater number of mammalian mutants. We have evaluated the base analog 5-Azacytidine (5AC), the anticancer drug, U73,975 and the human carcinogen, Treosulphan, as well as the suspect active metabolite of treosulphan, diepoxy butane (DEB), as mutagens in AS52 cells. All are potent mutagens and mutational spectra have been generated. Among the point mutations induced by 5AC, there is a substantial bias (>90%) for CG>GC transversions. These data suggest a direct role for 5AC as a direct acting mutagen, most likely through the generation of a 5AC:C mispairing during replication. The drug U73,975 is a less toxic analog of the widely studied antitumor drug, CC-1065. In vitro studies of both drugs have revealed preferred DNA binding sequence motifs. Of 125 U73,975-induced mutants analyzed, 44 (35%) are putative point mutants and 81 (65%) are deletions as analyzed by PCR. Among the point mutants, a hot spot for mutations is observed at a site in the gpt gene containing 3-overlapping consensus drug binding sequences. Treosulphan and DEB induce both deletion mutations (70%) and point mutations (30%). These data suggest that Treosulphan and DEB can induce mutations in mammalian cells via at least two mechanistic pathways. DNA sequence analysis is underway to compare point mutational spectra resulting from treosulphan or DEB treatment. All of these studies are designed to provide insight regarding the mechanisms by which these agents affect genomic integrity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-Z1038-10 ETB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chemical Metabolism and Disposition

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dr. H. B. Matthews Research Chemist ETB NIEHS
Others: Dr. L. T. Burka Research Chemist ETB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.3

PROFESSIONAL:

0.4

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Characterization of toxicity is fundamental to an assessment of human health risks resulting from exposure to chemicals. Basic to any characterization of chemical toxicity are studies of the fate and mechanisms of toxicity of chemicals of interest in one or more mammalian species. Such studies are designed to provide both applied knowledge of the fate of chemicals in the intact animal in support of toxicity tests conducted by the National Toxicology Program and basic knowledge of mechanisms of chemical toxicity. Each study is designed to address the impact of one or more factors such as dose, age, sex or route of exposure on the toxicity of the chemical(s) studied and the significance of this data to assessments of human health risks. Studies conducted during the current year have addressed the fate and mechanisms of toxicity of tris(2-chloroethyl)phosphate (TRCP), a flame retardant, used in many consumer products. TRCP was recently reported by the NTP to be a kidney carcinogen in rats. Approximately 50% of male rats exposed to 88 mg/kg for two years developed adenomas in renal tubules located in the outer margin of the medulla. Only 10% of female rats receiving the same dose developed adenomas in the same area. Studies currently underway are designed to determine the sex-related variations such as metabolism, clearance and concentration dependent toxicity which may account for the sex-dependent differences in carcinogenic responses observed in the bioassay. Results of these studies indicate that subtle differences in metabolism and dose in the target organ may account for the sex-dependent variations in carcinogenicity observed in the chronic studies. In another study, the combined toxicity of cimetidine, the active ingredient in the antiulcer drug Tagamet, and the pesticide diazinon is being investigated in an effort to determine if simultaneous exposure to these two compounds might enhance the toxicity of diazinon. Results of these studies indicate that diazinon is very rapidly metabolized and that simultaneous exposure to cimetidine has little effect on the half-life of diazinon, but does alter the ratio of some metabolites formed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21050-09-ETB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Evaluation of microencapsulation as a means to administer chemicals in feed

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.P. Dieter Physiologist ETB NIEHS

Others: M.R. Elwell Pathologist ETB NIEHS
T.E. Goehl Chemist CCB NIEHS

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Experimental Toxicology Branch

SECTION

General Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.6

PROFESSIONAL:

0.4

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrevoked type. Do not exceed the space provided.)

Microencapsulation is a process used to enclose volatile or chemically reactive compounds in starch microspheres so that long-term toxicity feeding studies can be conducted and to overcome potential palatability problems. Studies with microencapsulated citral in feed were compared with chemical administration by gavage in both sexes of F344 rats and B6C3F1 mice. Palatability was unaffected and toxic responses such as body and organ weight and tissue lesions occurred in the rodents, irrespective of route of exposure, indicating that microencapsulation could be used to replace the more costly and less desirable route of gavage administration in long-term studies. Higher doses of citral could be utilized after microencapsulation, resulting in maximization of the detection of potential toxic or carcinogenic responses. Further short-term toxicity studies to compare microencapsulated cinnamaldehyde, a volatile chemical, with chemical administration by gavage, are underway in both sexes of F344 rats and B6C3F1 mice. These studies will be used to determine target organ toxicity, and to select doses for longer-term studies by feeding the microencapsulated chemical, presuming this preferred route of administration can be utilized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21075-09 ETB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Xenobiotic Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. T. Burka	Chemist	DTRT	NIEHS
OTHERS:	H. B. Matthews	Supervisory Chemist	DTRT	NIEHS
	Robert Griffin	IRTA Fellow	DTRT	NIEHS
	Kelly Dix	Predoc IRTA Fellow	DTRT	NIEHS
	Adrian Phillips	Chemist	DTRT	NIEHS
	Mike Sanders	Biologist	DTRT	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

3.95

PROFESSIONAL:

1.95

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Understanding the metabolism, distribution, and elimination of chemical xenobiotics is often critical to an appreciation of their toxic effects. Extrapolation of results from animal testing to possible human health effects requires knowledge of metabolic pathways and of in vivo kinetic behavior. Investigation of mechanistic aspects of metabolic processes allows greater understanding of how metabolism of a xenobiotic might lead either to detoxification or to a reactive metabolite with greater toxicity. As more is learned about mechanisms of metabolism, more accurate predictions of possible metabolic pathways for new compounds should be possible. This group carried out studies on oxazepam (OXM), tris(2-chloroethyl) phosphate (TRCP), and ethoxyquin (EQN) in the past year. In an NTP study OXM, a Valium analog, has been found to increase the incidence of liver tumors in mice. Induction of OXM metabolism by pretreatment with OXM results in increased glucuronidation and a shift from fecal to urinary elimination. This altered elimination pattern closely resembles that found in man. Pharmacokinetics of TRCP, a flame retardant, were investigated in anesthetized male rats using conventional jugular vein cannulation and microdialysis coupled with mass spectrometry. All pharmacokinetic parameters determined were significantly different for the two methods at the $p=0.05$ level. The antioxidant EQN was rapidly absorbed, metabolized and excreted in urine as sulfate or glucuronide conjugates following oral administration to either rats or mice. Bile contained three glutathione conjugates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201-ES-21080-08 ETB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Magnetic Resonance Imaging of Rodent Tissues

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. R. Maronpot Veterinary Pathologists ETB NIEHS

COOPERATING UNITS (if any)

Department of Radiology, Center for In Vivo Microscopy, Duke Medical Center, Durham, NC

LAB/BRANCH

Experimental Toxicology Branch

SECTION

Cancer Genetics and Molecular Pathology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Magnetic resonance imaging techniques provide the opportunity to visualize internal organs at microscopic resolution in live animals and to study the effects of environmentally important chemical agents on perturbations in tissues and organs. Advanced techniques continue to be developed by Dr. G. Allan Johnson of Duke University permitting tissue resolution less than 50 microns in live animals and resolution less than 20 microns in fixed pathology specimens. Recent efforts have focused on obtaining MR images of fixed pathology specimens in which tissue damage is present. The MR images provide an alternative type of 'special stain' in that the proton images show structural details not present in conventionally stained sections. In addition, three-dimensional study and quantitation of findings is facilitated in acquired MR images of these tissue specimens. Current efforts involve implantation of rf coils on the liver surface of rats and collection of multiple high resolution images before, during and after treatment with known hepatotoxic agents. These studies show great promise as a means of monitoring dynamic tissue changes in live animals following exposure to potentially toxic environmental agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21084-07 ETB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Association of Chemically Induced Cell Proliferation and Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Dr. B. I. Ghanayem	Toxicologist	ETB	NIEHS
Others:	Dr. H. B. Matthews	Research Chemist	ETB	NIEHS
	I. Sanchez	Biologist	ETB	NIEHS
	Dr. M. Elwell	Vet. Pathologist	ETB	NIEHS
	Dr. R. R. Maronpot	Vet. Pathologist	ETB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

In a chronic study conducted by the National Toxicology Program (NTP), gavage administration of 100 or 200 mg ethyl acrylate (EA)/kg to F344 rats and B6C3F1 mice resulted in a significant dose-dependent increase of squamous cell papillomas and carcinomas in the forestomach (FS), with no increase in the incidence of tumors at any other site. Current studies were designed to investigate the temporal relationship between cell proliferation and carcinogenicity. Work in this laboratory indicated that carcinogenic doses of EA administered by gavage for 3 or 6 months resulted in a sustained increase in FS epithelial cell proliferation (hyperplasia) for as long as exposure to EA continued. However, hyperplasia regressed to background levels and resulted in no neoplastic lesions in 19 and 15 months of recovery, respectively. On the other hand, EA administration to male F344 rats, 5 days/week, for 12 months resulted in development of FS papillomas (2/5) after two months post treatment recovery. Further, rats treated for 12 months and allowed 9 months recovery developed squamous cell carcinomas (3/13) and papillomas (1/13). No significant elevation in liver CP or neoplasia was seen in any of the rats included in this study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21097-06-ETB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of chemical myelotoxicity using an in vivo leukemia transplant model

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M.P. Dieter	Physiologist	ETB	NIEHS
Others:	M.R. Elwell	Pathologist	ETB	NIEHS
	J.A. Garnett	Biol. Tech.	ETB	NIEHS

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Experimental Toxicology Branch

SECTION

General Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.6

PROFESSIONAL:

0.4

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

A cell transplant model for F344 rat leukemia was developed to investigate the mechanism of action of carcinogens, and to corroborate data from 2-year studies when necessary. The hypothesis that chemicals containing the dimethyl ester phosphonic acid group were potential leukemogens was tested further with trichlorfon, which was shown to accelerate the process of carcinogenesis; further studies with pesticides containing the diethyl dithiophosphate ester group are underway using the leukemia transplant model to determine the specificity of this tumor induction. The pesticide atrazine is also being investigated in this short-term transplant model since it is suspected to be associated with leukemia and lymphoma in farm workers. Studies of the mechanism of action of glycol alkyl ethers continued, and it was shown in the leukemia transplant model that neither the aldehyde or acid metabolites of ethylene glycol monomethyl ether were responsible for the anti-leukemic activity consistently exhibited by the parent chemical. Further studies with ethylene glycol monomethyl ether acetate showed that this chemical was more effective than the parent compound in extending the tumor latency period in the leukemia transplant model. Mononuclear leukemia cells separated from spleens of leukemic rats at various intervals following leukemia transplantation were used to evaluate the role of selected protooncogenes during the progression of carcinogenesis. The pattern of expression or inactivation of FMS, BCR, ABL, and SRC, protooncogenes involved in differentiation and proliferation of leukocytes, was compared in leukemic cells from spleens of untreated leukemic transplant recipients with those from recipients treated with ethylene glycol monomethyl ether to determine the potential role of these protooncogenes during the progression or inhibition of the carcinogenic processes. In vitro tests were also conducted with leukemic cell cultures from F344 rats, L1210 mouse leukemia cells, and HL60 human leukemia cells, which indicated that the parent compound but not the acid metabolite reduced the numbers of growing cells after addition of micromolar concentrations of ethylene glycol monomethyl ether.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21106-05 ETB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TERMINATED: September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

In situ Protocols for Mammalian Cell Mutagenesis Assays

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: William J. Caspary Chemist ETB NIEHS

Others: Diane Daston Biologist ETB NIEHS

COOPERATING UNITS (if any)

Dr. Collette Rudd, SRI International

LAB/BRANCH

Experimental Toxicology Branch

SECTION

General Toxicology Group

INSTITUTE AND LOCATION

NIH, NIEHS, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.7

PROFESSIONAL:

0.3

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Evaluation of the mutant fraction using standard mutagenesis assays can be inaccurate because mutant and wild-type cells are usually mixed during the expression phase. If some mutant progenitors or mutants grow more slowly than the wild-type cells during the expression period, there will be a decrease in the mutant to wild-type ratio and the mutant fraction will not accurately represent the number of mutational events that occurred. In order to accurately quantitate the mutagenic response of the cells, we have developed an in situ procedure which segregates and immobilizes cells during expression. In these experiments, we plated L5178Y tk⁺/mouse cells in semisolid medium immediately after treatment. The cells grew and formed microcolonies. We added the selective agent, TFT, as an overlay at specified times, permitting only TFTr cells to survive. In this procedure, each mutation is captured as an individual colony; consequently, the measured mutation fraction accurately reflects the mutational events that occur at the selected locus. In addition, the induced mutant colonies arising in the agar are the result of independent mutational events. We have developed the in situ assay protocol for the L5178Y cell line and showed that the spontaneous mutation rate measured in the in situ assay was 50-fold greater than when the cells expressed the phenotype in suspension. We evaluated the effect of chemical treatment on the mutation rate as a function of the time to TFT addition and generated expression curves for 6 compounds. The numbers of TFTr colonies produced at various times after treatment indicated that chemically-treated cultures had higher mutation fractions than the solvent controls. These results document the feasibility of quantitating induced mutation rates using the in situ protocol. This approach has also been applied to AS52 cell mutation assays at the gpt gene. We believe that this method should be considered for routine evaluation of the potential mutagenic effects of chemicals in mammalian cells in vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21109-05 ETB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of 2-Butoxyethanol Induced Hematotoxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. I. Ghanayem	Toxicologist	ETB	NIEHS
Others:	I. M. Sanchez	Biologist	ETB	NIEHS
	C. Wall	Biol. Aid	ETB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrevoked type. Do not exceed the space provided.)

Early work demonstrated that 2-Butoxyethanol (BE) causes acute hemolytic anemia in rats and that metabolic activation of BE to butoxyacetic acid (BAA) is a prerequisite for the development of anemia. In an attempt to assess the potential health risks of humans exposed to BE, studies were designed to determine the in vitro effect of BAA on RBCs from 10 mammalian species. Blood samples from each mammalian species were incubated with BAA at a concentration of 0, 1, or 2 mM and kept at 37°C in a gently shaking water bath. Complete blood counts were measured at 0, 1, 2 and 4 hr. BAA caused a time- and concentration-dependent increase in MCV and HCT (followed by hemolysis in certain species), in rats, mice, hamsters, rabbits, and baboons. In contrast, blood from pigs, dogs, cats, guinea pigs, and humans was minimally affected by BAA. These results were confirmed in guinea pigs and rats in vivo. Gavage administration of 250 mg BE/kg to rats resulted in increased MCV and HCT followed by hemolysis. Identical treatment with BE resulted in no significant change in these parameters in guinea pigs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201-ES-21119-04-ETB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism and Mutagenicity of Mutagenic Noncarcinogens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michael L. Cunningham Toxicologist ETB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.1

PROFESSIONAL:

1.1

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Research in this laboratory is directed to investigations of mechanisms whereby compounds which exhibit mutagenicity in short-term assays such as the Ames/Salmonella test may fail to induce carcinogenesis in rodents under conditions of the NTP 2-year bioassay. This nonconcordance between short-term tests and NTP bioassay results weakens the use of in vitro assays for prediction of in vivo carcinogenicity. The results of this research indicate that the mutagenic noncarcinogens consistently fail to induce cell proliferation in vivo. Similar work in this laboratory with positive carcinogens, either mutagenic or nonmutagenic, demonstrated each of these chemicals did induce cell proliferation, and further, the cell proliferation was associated with the site of carcinogenesis. We have studied 17 chemicals to date (15 in-house; 2 on contract) which vary considerably in their structural classes. A series of 5 organophosphate compounds demonstrated that the potent renal carcinogen tris(2,3-dibromopropyl)phosphate produces cell proliferation in the renal outer medulla, the site of renal tumors in the bioassay. Noncarcinogens in this class did not produce cell proliferation in liver or kidney. A 90 day feeding study was conducted in-house on the anxiolytic agent Oxazepam which combined cell proliferation measurements with clinical chemistry and pharmacokinetics. Our data demonstrated that this compound may be carcinogenic in B6C3F1 mice due to its ability to produce cell proliferation by a mitogenic mechanism. The results of the research conducted in this laboratory suggest a positive correlation between cell proliferation and carcinogenesis for these chemicals, irrespective of their mutagenicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21128-03 ETB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bile Acids as Indicators and Initiators of Hepatotoxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P:I Morrow B. Thompson Veterinary Medical Officer ETB NIEHS
Others: Rickie D. Fannin Biologist ETB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Toxicology Branch

SECTION

Clinical Pathology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

0.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

Metabolism of bile acids has been characterized in vivo and in vitro, the latter in a variety of systems including isolated perfused liver and transformed and normal hepatocytes. We have been exploring the use of precision-cut slices of rat liver to study bile acid metabolism. Initially, secretion of individual bile acids by slices from control rats and rats treated with α -naphthylisothiocyanate (ANIT) or clofibrate was investigated. In each study, bile acids were added to the medium to examine effects on conjugation, metabolism and secretion of bile acids by slices. Bile acids in media were measured using a HPLC/enzymatic method with fluorescence detection. In these experiments (6 to 7 hr incubations in Williams' E medium), secretion of total bile acids by slices from control rats ranged from 160 to 447 pmol/mg protein/hr. These rates are 3 to >30-fold higher than those reported for cultured hepatocytes and compare favorably with those for isolated perfused liver. Secretion rates of individual bile acids from slices from rats treated with ANIT were significantly increased for tauro-a-muricholate, tauroallocholate, taurocholate (strong trend), tauroallochenodeoxycholate, and taurochenodeoxycholate, and decreased for taurohyodeoxycholate. The addition of b-muricholate to the medium increased the secretion of tauro-b-muricholate by approximately 10-fold and increased the formation of an unknown bile acid, identified as D22-tauro-b-muricholate by NMR spectroscopy, by 4-fold. Slices from rats treated with clofibrate had total rates of secretion that were approximately 3-fold less than those from control rats. These lower rates resulted from decreased secretion of glyco-b-muricholate, tauro-b-muricholate, D22-tauro-b-muricholate, taurohyodeoxycholate, and taurocholate. Decreased taurocholate secretion persisted even with the addition of taurine and glycine and/or cholate to the medium. These experiments demonstrate that liver slices can provide a valuable alternative to other in vitro systems for the study of bile acid metabolism and secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21129-03 ETB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Frozen Tissue Archives

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. R. Maronpot Veterinary Pathologist ETB NIEHS

COOPERATING UNITS (If any)

EPL, Inc. (Contractor for NTP Archives)

LAB/BRANCH

Experimental Toxicology Branch

SECTION

Cancer Genetics and Molecular Biology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

0.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Samples of rodent tumor or normal tissues from National Toxicology Program carcinogenicity studies, in in-house carcinogenicity studies, and from various other contract efforts continue to be collected and frozen for subsequent molecular biology studies. Approximately 12,000 samples have been frozen for use by various NIEHS investigators and over 1,200 of these samples have been or are in the process of being studied for oncogene and suppressor gene activities. All samples have been entered into a searchable data base and all tumors have been diagnosed and cataloged for orderly retrieval. Results from study of these samples is providing useful information for the interpretation of the biological significance of tumors in carcinogenicity bioassays, in understanding carcinogenesis, and in risk assessment for environmentally important chemicals. It is anticipated that this effort will continue over the next two years.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21130-03 ETB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Histogenesis of Pulmonary Neoplasms in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Darlene Dixon	Veterinary Pathologist	ETB	NIEHS
Others:	Alicia D. Bowser	Biologist	ETB	NIEHS
	Robert R. Maronpot	Veterinary Pathologist	ETB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Experimental Toxicology Branch

SECTION

Toxicologic Pathology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

1.25

OTHER:

1.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Studies designed to assess the histomorphological, ultrastructural histochemical and biochemical characteristics of altered cell types in pulmonary neoplasms of mice have been done. Over the past two years, we have evaluated the gross, histomorphological and ultrastructural features of pulmonary neoplasms in aged Strain A mice, B6C3F1 mice and also F344 rats using conventional light and electron microscopy. Automated lectin staining and two dimensional gel electrophoresis have been used to characterize the the glycoconjugate composition of nonneoplastic versus neoplastic cells, and malignant versus benign neoplasms in Strain A mice. Studies designed to evaluate the interaction of fibers and chemical carcinogens on pulmonary tumorigenesis are underway. In vitro studies to characterize the histogenesis of mouse lung tumors are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-Z1131-03 ETB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Chemically Induced and Spontaneous Neoplasms for Oncogene Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R.R. Maronpot Veterinary Pathologist ETB NIEHS

Others: Marshall Anderson Research Chemist LMT NIEHS

COOPERATING UNITS (if any)

Laboratory of Molecular Toxicology

LAB/BRANCH

Experimental Toxicology Branch

SECTION

Cancer Genetics and Molecular Pathology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Oncogene studies on spontaneous and chemically induced rodent neoplasms started 6 years ago in collaboration with molecular biologists in the Division of Biometry and Risk Assessment and are continuing. Emphasis has been on the patterns of oncogene activation in chemically induced versus spontaneous lung and liver tumors in rats and mice using samples from National Toxicology Program bioassays as well as from rodent studies designed to investigate strain susceptibility to genotoxic and nongenotoxic chemical carcinogens. Important information useful in interpreting bioassay results and in risk assessment has been generated from these studies and underscores the need to continue similar investigations. Current work has been expanded to investigate the role of suppressor genes in rodent carcinogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201-ES-21132-03 ETB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Image Analysis, Quantitative Morphometrics and Cell Proliferation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. R. Maronpot Veterinary Pathologist ETB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Toxicology Branch

SECTION

Cancer Genetics and Molecular Pathology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.2

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Special emphasis has been given to development of methodologies and study of cell proliferation during the past year. Immunohistochemical procedures for staining S-phase cells labeled with bromodeoxyuridine have been optimized and are in routine use in rats and mice. We have established an immunohistochemical methodology to identify cells expressing proliferating cell nuclear antigen (PCNA) and have successfully applied this procedure to archival specimens that were fixed in formalin and embedded in paraffin. This allows us to take advantage of the National Toxicology Program tissue archives and the thousands of samples available from past studies. A computer assisted program has been developed to automate counting of cell proliferation and simultaneously obtain ploidy data on rodent liver samples. Standard morphometric procedures are being used to study the progressive development of preneoplastic lesions in mouse liver. This work will continue for the next two years.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21138-03 ETB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Comparative Metabolism, Disposition, and Toxicity of Aliphatic Nitriles

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Dr. B. I. Ghanayem	Toxicologist	ETB	NIEHS
Others:	I. Sanchez	Biologist	ETB	NIEHS
	Dr. L. T. Burka	Chemist	ETB	NIEHS
	Dr. A. E. Ahmed	Prof., Univ of Tx	ETB	NIEHS

COOPERATING UNITS (if any)

University of Texas

LAB/BRANCH

Experimental Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Methacrylonitrile (MAN) is used in the production of plastics and elastomers and is structurally related to the known carcinogen, acrylonitrile (AN). Present work was undertaken to investigate the biological fate of MAN in male F344 rats. Following gavage administration, MAN was rapidly absorbed from the GI tract and distributed to all major tissues. After administration of 1.15, 11.5, or 115 mg/kg, 2-14C-MAN is primarily eliminated in the expired air. Sixty to 70% of the low and medium doses were exhaled as 14CO₂ in 72 hr compared to 25% of the highest dose. While 40% of the high dose was expired as organic volatiles in 72 hr, only 9-12% of the low and medium doses were exhaled as such. It is therefore apparent that saturation of MAN metabolism occurs at the high dose. HPLC analysis of expired organic volatiles from MAN-treated rats showed that it contained two components which were identified as unchanged MAN and acetone. The MAN/acetone ratio was directly proportional to dose and decreased as a function of time. Urinary excretion accounted for 20-30% of all MAN doses within 72 hr after dosing. Administration of MAN (115 mg/kg) in oil resulted in the death of rats within 24 hr after treatment. Monitoring the fate of MAN in these rats prior to death showed that a significantly higher % of the dose was eliminated in urine and expired air. Expired air from these animals contained significantly more acetone and less unchanged MAN than rats receiving MAN in water. Comparison of the metabolism and disposition of MAN in F344 and SD rats showed minor differences between the two strains. Further, administration of MAN to SD rats in oil caused minimal change in MAN disposition and had no lethal effect on this strain of rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21139-03 ETB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Mechanisms of Mutagenesis and Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: William J. Caspary Chemist ETB NIEHS

COOPERATING UNITS (if any)

Dr. Ken Tindall, ECMB

Drs. D. Schiffmann and H Stopper, University of Würzburg

LAB/BRANCH

Experimental Toxicology Branch

SECTION

General Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2.5

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

The cytidine analog, 5-azacytidine (5AC) alters gene expression. We examined the mutagenic activity of a series of cytidine analogs, including 5AC, in L5178Y mouse cells and found that their mutagenic potency correlated with their reported ability to induce differentiation C3H10T1/2 cells. We are analyzing 5AC induced mutants in AS52 cells. 5AC was an effective mutagen from 0.5 to 5.0 µg/ml. 6TGr-AS52 clones were induced for molecular analyses with 3.0 µg/ml 5AC. 6TGr clones were isolated and DNA was prepared for polymerase chain reaction (PCR) and sequence analysis. The frequency of deletions and putative point mutations was determined by PCR analysis. Of 149 6TGr clones analyzed, 134 (90%) were putative point mutants (wild-type PCR product observed) and 15 (10%) were deletion mutants (alteration or loss of the gpt PCR product). Of the 134 putative point mutants, 53 (36% of the total mutants examined) showed base mutations in the structural region of the gene. 5AC induced micronuclei in L5178Y cells at concentrations at which it is mutagenic. The induced micronuclei contained chromosomal fragments as evidenced by our inability to observe CREST staining of kinetochores. 5AC did not induce micronuclei via interference with chromosome morphology during prophase or metaphase and it affected mitosis only after the cells entered anaphase. Chromatid bridges were observed in anaphase that extended into telophase and even into interphase. The compound induced micronuclei if added while the cells were in S phase. 5AC had no effect on the spindle formation and morphology and that the metaphase plate looked normal. Some of the chromosomes in cells treated with 5AC were not able to separate during mitosis, resulting in tetraploidy. This occurred during the first mitosis after hypomethylation where one of the sister chromatids would be fully methylated and the second hemimethylated. It appears that maximal endoreduplication occurred during replication of hemimethylated DNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21143-03-ETB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Toxicology Studies of Lead

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.P. Dieter Physiologist ETB NIEHS

Others: M.R. Elwell Pathologist ETB NIEHS
 R.S. Chhabra Toxicologist ETB NIEHS
 H.B. Matthews Toxicologist ETB NIEHS

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Experimental Toxicology Branch

SECTION

General Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.6

PROFESSIONAL:

0.4

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A study was conducted to compare the toxicity and bioavailability of lead in male F344 rats exposed to pure lead salts or to lead ore from Skagway, AK. The chemicals were fed to rats for 30 days. At study termination lead concentrations in blood, bone, kidney, and brain, and urinary aminolevulinic acid were measured. The soluble lead salts, lead oxide and lead acetate, accumulated to a greater degree in bone and soft tissues than the insoluble lead sulfide or the lead ore from Skagway, AK. There was a strong correlation between dose and bone lead concentration for either soluble or insoluble lead samples when the data were graphed on a long-linear scale. Use of the aminolevulinic acid biomarker was ineffective at bone lead concentrations below 20 µg/g. Blood lead concentrations were ~ 80 µg/dl in rats fed the soluble lead salts, and ~ 10 µg/dl in rats fed lead sulfide or lead ore. The highest lead concentrations in soft tissue occurred in kidney, followed by brain. Rats fed soluble lead salts accumulated about 10-fold higher lead concentrations in the soft tissues compared to those fed lead sulfide or lead ore. However, detectable and potentially toxic concentrations of lead accumulated in a dose-related fashion in kidney and brain of rats fed the less soluble lead samples, and to a higher degree in rats fed lead ore compared to those fed lead sulfide. These data suggest that longer exposure periods might result in continual and greater accumulations of lead. Kidney lesions occurred in rats dosed with 30 or 100 ppm lead acetate but not in other treatment groups. Lead ore samples from three other mining sites, Alaska, Missouri, and Colorado, are being fed to rats for longer periods (90 days) to compare the bioavailability and toxicity of ores from different geographical areas. Assays of blood δ-aminolevulinic acid dehydratase enzyme activity, a sensitive and specific biomarker for lead toxicity, and determinations of lead in blood, kidney, and brain are being conducted at 30, 60, and 90d exposure. Data from the 30d samples indicate that at 100ppm in the feed two samples, Alaskan lead ore and Missouri lead ore, significantly inhibited blood ALAD enzyme activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21159-02 ETB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Induction of Glutathione S-Transferase-Pi in Cultured Rat Liver Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Po C. Chan

Biologist

ETB

NIHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Toxicology Branch

SECTION

General Toxicology Group

INSTITUTE AND LOCATION

NIHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Glutathione S-transferase-pi (GST-P) is detected in rat liver altered hyperplastic foci and hepatocellular carcinoma and is considered the most reliable biomarker in rat hepatocarcinogenesis. Ito and coworkers (Toxicologist, 12:205, 1992) have used an in vivo assay system for GST-P induction for identifying carcinogenicity of 198 chemicals and reported it to be highly effective for identifying hepatocarcinogens but not for nonhepatocarcinogens. The reason for the ineffectiveness of Ito's assay system to identify nonhepatocarcinogens is probably because most of the nonhepatocarcinogens are direct acting or being activated at the site of application. We have used a cultured rat liver cell (GP6 cell line) system to study induction of GST-P by hepatocarcinogens, nonhepatocarcinogens, and noncarcinogens. GP6 cells at 100,000 cells/dish in Richter's medium +10% fetal calf serum and gentamycin were exposed to dimethylsulfoxide (DMSO) at 0, 0.1, 0.2, or 0.4%, phenobarbital (PB) at 1.5 mM, diethylnitrosamine (DEN) at 16 μ l/ml, or dimethylbenzanthracene (DMBA) at 0.2 μ g/ml in the presence of rat liver S9. The cells were refed with fresh medium containing the chemical every 3-4 days and subcultured weekly. Cytosolic GST-P activity was assayed by spectrophotometry using ethracrynic acid as substrate. Compared with controls, GST-P activity increased significantly in GP6 cells exposed to PB, DEN, or DMBA for 3-5 weeks. DMSO at up to 0.4% had no effect on GST-P activity. The results showed that the nonhepatocarcinogen DMBA, along with the hepatocarcinogens, DEN and PB, were effective in elevating GST-P activity in GP6 cells whereas the noncarcinogen, DMSO, was ineffective. This in vitro system using GST-P as a marker may be useful for identifying carcinogens including nonhepatocarcinogens since direct acting carcinogens such as DMBA can be studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21163-02 ETB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Toxicokinetics and Toxicodynamics of Xenobiotics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Dr. Karen Demby	Staff Fellow	ETB	NIEHS
Others:	Dr. Robert Griffin	IRTA Fellow	ETB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.50

PROFESSIONAL:

1.25

OTHER:

1.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unvulsed type. Do not exceed the space provided.)

Toxicokinetic and toxicodynamic studies of xenobiotics are a useful extension of traditional chemical disposition studies in a number of ways. These studies provide a method by which quantitative predictions of systemic exposure to chemicals may be made. They also increase generalization of information obtained from toxicity studies. This generalization permits prediction of toxicity in species other than those tested, including man. The toxicokinetics of two chemicals, methacrylonitrile (MAN) and oxazepam (OXAZ) are currently being investigated in this laboratory. The toxicokinetics of MAN was compared in male Fischer 344 and Sprague-Dawley rats. This comparison was made to determine if differences in toxicity between strains was related to different levels of exposure to MAN or MAN-derived cyanide. The data suggest that the differences in toxicity between strains are not wholly related to exposure to either MAN or cyanide and is more likely attributable to acetone cyanohydrin. The kinetics of oxazepam (OXAZ) is being studied in rats and mice. The effect of prefeeding with OXAZ (2500 ppm) on its own elimination was investigated in the F344 rat. Results show that its elimination is increased relative to controls following 14 days of prefeeding. In contrast, following 28 days of prefeeding, OXAZ elimination is comparable to control values. The increase in elimination at 14 days was not correlated with changes in cytochrome P450 or cytochrome b5. It is speculated that biliary elimination is increased following 14 days of feeding. A physiologically-based pharmacokinetic model is being developed to describe the kinetics of OXAZ in the B6C3F1 mouse. This model will be used to extrapolate results of toxicity and carcinogenicity studies in mice to predict risks associated with human exposure to OXAZ.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21009-11 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Reproductive Effects in Males Exposed to Environmental Chemicals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Co-P.I.'s:	Robert E. Chapin, Ph.D.	Toxicologist	STB	NIEHS
	Warren W. Ku, Ph.D.	Toxicologist	STB	NIEHS
Others:	Robert N. Wine	Biologist	STB	NIEHS
	Martha W. Harris	Biologist	STB	NIEHS
	Janet Allen	Biologist	STB	NIEHS
	Eric Haskins	Biologist	STB	NIEHS

COOPERATING UNITS (if any)

Comparative Medicine Branch, DIR

LAB/BRANCH

Systems Toxicity Branch

SECTION

Developmental and Reproductive Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NTP, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

1.8

PROFESSIONAL:

0.7

OTHER:

1.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Work continued on the boric acid testicular effect. A dose-time study was performed which defined new relationships between endpoints commonly used for testicular assessment. Atrophy was found in the long-term dosed animals. Despite a short biologic half-life and complete clearance from blood in 3-4 days, no testicular recovery was seen. Bone was found to be a long-term depot for boron, but circulating levels were not significantly raised over controls in animals recovering from boron exposure. In vitro studies identified an adverse effect on DNA synthesis in spermatogonia, an effect which was replicated in vivo. A collaborative project will evaluate whether exogenously added paracrine factors will be able to stimulate recovery of spermatogenesis in these atrophic testes. This could ultimately lead to a clinical product that might improve spermatogenesis for sub-fertile males.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21031-08 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Computer Simulation of Inhalation Exposures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: M. P. Moorman Engineer STB NIEHS

Others: D. L. Morgan Toxicologist STB NIEHS

COOPERATING UNITS (if any)

METI

LAB/BRANCH

Systems Toxicity Branch

SECTION

Respiratory Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unbolded type. Do not exceed the space provided.)

Computer simulations of physiologically based pharmacokinetic models are being used to study the uptake and metabolism of compounds administered by inhalation. The application of these models to specific compounds requires two types of compound specific data: tissue partition coefficients and metabolic constants. Systems for making these measurements have been developed by adapting the designs used by other laboratories. Tissue partition coefficients are determined by measuring the partitioning between tissue homogenates and the headspace in sealed vials. Metabolic rate constants are measured by monitoring the removal by the test animals of the compound from the atmosphere of a sealed recirculating exposure system. A computer simulation of the test animals and the exposure system is used to estimate the metabolic rates from these measurements. The values of metabolic parameters are determined by adjusting the metabolic constants of the simulation until the simulation results agree with the measured data.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21046-09 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Postnatal Toxicology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Frank Kari, Ph.D. Toxicologist STB NIEHS

Others: Robert Weaver Biologist STB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Systems Toxicity Branch

SECTION

Developmental and Reproductive Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.8

PROFESSIONAL:

0.8

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unswollen type. Do not exceed the space provided.)

Our studies have identified chemicals which are excreted into milk in greater concentrations than predicted by established pharmacokinetics principals. Our current work suggests that active/facilitated secretion mechanisms may be responsible for the unpredictable milk/plasma ratios that are observed. We are working to characterize the mechanisms responsible for the non-diffusion mode of xenobiotic accumulation in experimental models and to explore the relevance of these observations in humans. Established in vivo and in vitro models are being used to identify other potential substrates for facilitated/active uptake by mammary secretory cells and to characterize the nature of the transport mechanisms. This work can be expanded to include human studies to provide better quantitative estimates of the propensity for chemicals to be transported to nursing infants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21089-06 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Action of Testicular Toxicants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Co-P.I.'s: Robert E. Chapin, Ph.D. Toxicologist STB NIEHS
Jerrold J. Heindel, Ph.D. Toxicologist STB NIEHS

Others: Cassandra J. Powell Biologist STB NIEHS
Robert N. Wine Biologist STB NIEHS

COOPERATING UNITS (if any)

Comparative Medicine Branch, DIR

LAB/BRANCH

Systems Toxicity Branch

SECTION

Developmental and Reproductive Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

1.9

PROFESSIONAL:

0.7

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Work continued on both new methods development and ongoing hypothesis testing. The mechanism of action of phthalates was investigated, using animals of different ages to evaluate possible effects on the FSH receptor. Inhibition of the affinity of FSH binding was observed; the mechanism of this effect is currently under investigation. New methods were developed to evaluate testicular toxicity. Digital fluorescent imaging is being developed to assess early biochemical changes in toxicant-exposed cells. Significant progress was made in bringing this technique on-line. Two-dimensional gel electrophoresis methods were optimized to evaluate toxicant-induced changes in gene expression and post-translational modification. These methods are currently being applied to determine the mechanism of action of several Sertoli cell toxicants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21090-07 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Mechanisms of Arsine Gas and Gallium Arsenide Toxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Daniel L. Morgan	Toxicologist	STB	NIEHS
Others:	B.A. Schwetz	Supervisory Toxicologist	STB	NIEHS
	M. P. Moorman	Engineer	STB	NIEHS

COOPERATING UNITS (if any)

METI
University of Maryland

LAB/BRANCH

Systems Toxicity Branch

SECTION

Respiratory Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

0.05

PROFESSIONAL:

0.05

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unnumbered type. Do not exceed the space provided.)

Studies have been conducted to evaluate the acute and short-term toxicity of arsine gas. Fisher 344 rats, B6C3F1 and C57BL/6 mice, and Syrian golden hamsters have been exposed to arsine gas at concentrations of from 10 ppb to 50 ppm for periods ranging from .5 hr to 90 days. All groups exposed to a single 6 hr exposure of 25 ppm experienced 100% mortality, while those exposed to 5 ppm for four weeks or 2.5 ppm for 13 weeks showed no overt signs of toxicity. Urine samples from these studies showed increased levels of coproporphyrin and 7 and 8 carboxyl uroporphyrin. These data suggest that alterations in the heme biosynthetic pathway as reflected in increases of specific species of urinary porphyrins may be used as early biological indicators of ongoing arsine toxicity. A method has been developed to improve the measurement of specific porphyrin species in rodent urine to further refine this model. Tissues from these exposures are being analyzed for arsenic content to provide a measure of the tissue dose for specific exposure regimens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 21110-05 STB	
PERIOD COVERED October 1, 1991 to September 30, 1992			
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Developmental Immunotoxicity Studies of Inbred Mice			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)			
P.I.:	Jerrold J. Heindel	Toxicologist	STB NIEHS
Others:	Steven D. Holladay	IRTA	STB NIEHS
	Michael I. Luster	Research Microbiologist	STB NIEHS
	Benny L. Blaylock	IRTA	STB NIEHS
COOPERATING UNITS (if any) Immunotoxicology Group, STB Comparative Medicine Branch, DIR			
LAB/BRANCH Systems Toxicity Branch			
SECTION Developmental and Reproductive Toxicology Group			
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709			
TOTAL STAFF YEARS: 1.2		PROFESSIONAL: 1.1	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☐ (a1) Minors ☐ (a2) Interviews			
SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.) Chemicals such as 2,3,7,8-tetrachlorodibenzo-p-dioxin and diethylstilbestrol, and naturally occurring toxins such as the tricothecene mycotoxin, T2 toxin, adversely affected immunologic function in offspring following treatment of pregnant mice over various periods of gestation. Studies are in progress to explore the relationship between developmental immunotoxicity and subsequent adult immune function. These studies are, in particular, addressing the following question: Do modulations in lymphocytic surface antigens, induced by prenatal chemical exposure, result in functional immunologic deficits later in life? TCDD, DES, or T2 toxin are administered to pregnant C57B1/6 and B6C3F1 mice during gestation to establish the sensitive period for induction of immunologic deficits and to identify the initial lesion. Fetal T and B lymphocytes from the spleen and thymus, and subpopulations of these cells, are stained immunocytochemically to determine the morphological effects of TCDD, DES, and T2 toxin on lymphocytic surface antigens and their development. Fetal thymic organ culture is used to determine effects of exposure on subpopulations of immune cells. In addition, cell populations are evaluated by flow cytometry to determine quantitative changes. If changes in these surface markers persist beyond the age of four weeks in the prenatally exposed animal, functional tests, including mixed lymphocyte reaction, plaque assay, in vitro blastogenesis, cytotoxic T lymphocyte function, and colony forming unit assay, are conducted. TCDD was found to significantly decrease fetal thymic weight and cellularity, and to significantly alter normal patterns of intrathymic differentiation as defined by thymocyte expression of the cell surface antigens CD4, CD8, J11d, PNA, and TCR. Mice exposed to TCDD were found to have significantly depressed cytotoxic T lymphocyte activity at eight weeks postnatally. DES and T2 toxin have been found to produce fetal alterations similar to, but less severe than, that seen after TCDD.			

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21118-04 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Mechanism of Action of Ovarian Toxicants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Jerrold J. Heindel	Toxicologist	STB	NIEHS
Others:	Barbara Davis	Student (Ph.D.)	STB	NIEHS
	Jessica Sneedeen	Stay-in-School	STB	NIEHS

COOPERATING UNITS (If any)

Comparative Medicine Branch, DIR

LAB/BRANCH

Systems Toxicity Branch

SECTION

Developmental and Reproductive Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.9

PROFESSIONAL:

0.4

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is composed of two aspects: determination of the ovarian cell type which is the most sensitive to the action of the toxicant, i.e., the target cell, and then the determination of the mechanism of action of the toxicant on that particular cell. With regard to the first part, we have determined the ovarian target cell for cyclophosphamide (growing granulosa cells) and are determining the target cell for phthalate esters and glycol ethers.

Mechanistically we are examining the effect of phthalate esters on granulosa cell function (the target cell based on preliminary evidence) using granulosa cells cultured from DES-implanted immature rats (F-344). Mono(2-ethylhexyl)phthalate, the active metabolite of the phthalate ester di-(ethylhexyl)phthalate, inhibits FSH-stimulated cAMP accumulation in a time- and dose-dependent manner. MEHP also inhibits progesterone production, a cAMP dependent process. The action of MEHP appears to be at the level of the FSH receptor or receptor coupling to the adenylate cyclase. It does not involve activation of protein kinase C as the action of MEHP is not mimicked by phorbol esters. We are currently examining the effect of MEHP on granulosa cell ATP levels and mitochondrial function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21124-04 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of a Model to Study the Influence of Nutrition on-F344/N Rat Leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Frank Kari, Ph.D.	Toxicologist	STB	NIEHS
Others:	John French, Ph.D.	Physiologist	ECMB	NIEHS
	Steven Hursting	Biologist	STB	NIEHS

COOPERATING UNITS (if any)

Experimental Carcinogenesis and Mutagenesis Branch, DTRT

LAB/BRANCH

Systems Toxicity Branch

SECTION

Developmental and Reproductive Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.2

PROFESSIONAL:

0.2

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrevolved type. Do not exceed the space provided.)

We have demonstrated that corn oil gavage and feed restriction inhibit the progression of mononuclear cell leukemia in male but not female rats. We have developed a diffusion chamber culture technique which allows us to evaluate the effect of host factors on the proliferation and differentiation of mononuclear cells. Results from these experiments reveal that circulating factors can influence the in situ proliferation of mononuclear leukemia cells. Serum isolated from animals on anti-leukemic regimens (feed restricted, corn oil, gavage, etc.) is less able to promote leukemia cell proliferation in vitro than serum from normal animals. We have identified the growth hormone: insulin like growth factor I axis as being an important mediator of the feed restriction induced inhibition of mononuclear cell leukemia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21127-03 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Short-Term Comprehensive Reproductive and Developmental Toxicity Screen

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Robert E. Chapin, Ph.D. Toxicologist STB

Others: Martha Harris Biologist STB
 Eric Haskins Biologist STB
 Janet Allen Biologist STB
 E. Sidney Hunter, Ph.D. Toxicologist STB

COOPERATING UNITS (if any)

Comparative Medicine Branch, DIR
 Histology Laboratory, DTRT

LAB/BRANCH

Systems Toxicity Branch

SECTION

Developmental and Reproductive Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

0.2

OTHER:

1.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Work continued to focus on elements in the boron column of the periodic table. For indium trichloride, a dose-range finding study was performed, followed by the short-term test which identified developmental toxicity. A developmental toxicity study followed which showed fetal killing in the absence of malformations. A disposition study was performed to identify the levels of indium present in selected target and non-target tissues at the time of toxicity. Subsequent in vitro studies were performed that showed that indium is directly fetotoxic. A final study will assess the degree of absorption and body burden of indium after inhalation exposure (the most likely human route). Future studies will assess the toxicity of gallium trichloride and three photovoltaic III-V compounds (CuInSe₂, CuGaSe₂, and CdTe).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21135-03 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

The Effects of AIDS Therapeutics on the Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Gary J. Rosenthal	Toxicologist	STB NIEHS
Others:	M. Luster	Research Microbiologist	STB NIEHS
	E. Corsini	Guest Researcher	STB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Systems Toxicity Branch

SECTION

Immunotoxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

1.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

The Immunotoxicology Group is continuing the assessment of immunotoxicity of AIDS therapeutics. The ongoing objectives include: (1) to evaluate the potential adverse effects of promising AIDS therapeutics on systemic as well as local systems; (2) if possible, to examine potential mechanisms of toxicity (or therapeutic action, if unknown); (3) to relate these observed changes in immune function to clinicians and regulatory agencies so that improved treatment and monitoring may be facilitated.

Studies were performed in the following areas: a) descriptive immunotoxicity studies on pentamidine isethionate and related analogs on lung macrophages. The endpoints for these studies focus on cytokine production; b) mechanistic studies on the cellular and subcellular targets of these drugs; and c) immunopharmacologic examinations of pentamidine and in disease models.

The results of these studies demonstrated that following endotoxin administration, pentamidine has a protective and anti-inflammatory role both systemically and in the lung and suggests that inhibition of inflammatory cytokines such as TNF and IL-6 may be one mechanism operable in the therapeutic activity of the drug against P. carinii pneumonia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21136-03 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Toxicity Studies of Styrene and Related Chemicals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Daniel L. Morgan	Toxicologist	STB	NIEHS
Others:	B.A. Schwetz	Supervisory Toxicologist	STB	NIEHS
	M. P. Moorman	Engineer	STB	NIEHS

COOPERATING UNITS (if any)

METI

LAB/BRANCH

Systems Toxicity Branch

SECTION

Respiratory Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

0.65

PROFESSIONAL:

0.6

OTHER:

0.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unlined type. Do not exceed the space provided.)

Inhalation toxicity studies were designed to study the toxicity and potential carcinogenicity of styrene, alpha-methylstyrene, and divinylbenzene. Styrene was the first chemical in this series of related compounds to be studied. Styrene toxicity and metabolism have been studied extensively in rats; however, data for a second species is needed by regulatory agencies for risk assessment and setting standards. A series of experiments was conducted to characterize the toxicity of styrene in mice. Male B6C3F1 mice were considerably more susceptible than females to the lethal effects of styrene. Both sexes demonstrated a non-linear dose response where hepatotoxicity and mortality were greater at 250 ppm than at 500 ppm, even though the blood levels of styrene oxide, the presumed toxic metabolite, were higher at 500 ppm. In a study comparing susceptibility of different mouse strains to styrene toxicity, mortality was greatest in B6C3F1 mice, intermediate in Swiss mice, and lowest in DBA/2 mice. Although no DBA/2 mice died during styrene exposure, the incidence and severity of hepatotoxicity was similar in DBA/2 and B6C3F1 mice. Blood levels of styrene oxide were highest in B6C3F1 mice, intermediate in DBA/2 and lowest in Swiss mice. These data will be used to select the appropriate mouse strain and dose levels for two-year chronic toxicity and carcinogenicity studies of styrene in mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21137-03 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Mechanisms of Tetranitromethane Toxicity and Carcinogenicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Daniel L. Morgan	Toxicologist	STB	NIEHS
Others:	B.A. Schwetz	Supervisory Toxicologist	STB	NIEHS
	M. P. Moorman	Engineer	STB	NIEHS
	Tracy Futch	Biologist	STB	NIEHS

COOPERATING UNITS (If any)

METI

LAB/BRANCH

Systems Toxicity Branch

SECTION

Respiratory Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

1.00

PROFESSIONAL:

0.05

OTHER:

0.95

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Tetranitromethane (TNM) was recently found to be one of the most potent pulmonary carcinogens studied by the NTP, yet the toxic and carcinogenic mechanisms of TNM and related nitro-compounds are unknown. The objective of this project is to investigate the mechanisms(s) of tetranitromethane (TNM) pulmonary toxicity and carcinogenicity. Studies were conducted to investigate the biochemical reactions of TNM with nucleosides and DNA in vitro. Purified nucleosides were incubated with TNM in a buffered solution at different pHs for up to 24 hours and then analyzed by HPLC for reaction products. No altered nucleosides were detected, indicating that TNM does not react with purified nucleosides in this experimental system. The potential reaction of TNM with DNA was investigated by incubating TNM with purified herring sperm DNA in a buffered solution for up to 24 hours. After reaction, the DNA was hydrolyzed to individual nucleotides, and the hydrolysate analyzed by HPLC for altered bases. The only products detected were the four unchanged nucleotides, TNM, and nitroform (a breakdown product of TNM), indicating that TNM does not react with purified DNA. These data suggest that TNM may require metabolism to a reactive intermediate before binding to DNA and other macromolecules, and/or TNM interacts with DNA indirectly (e.g. by modification of histones). Studies to evaluate the potential metabolism of TNM by lung microsomes are in progress. These studies will provide the basis for subsequent mechanistic studies performed in cultured cells exposed to TNM.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21148-02 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Lung Immunotoxicology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Gary J. Rosenthal	Toxicologist	STB NIEHS
Others:	E. Corsini	Guest Researcher	STB NIEHS
	L. Kong	Guest Researcher	STB NIEHS
	M. Blazka	IRTA Fellow	STB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Systems Toxicity Branch

SECTION

Immunotoxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

The Immunotoxicology Group is continuing studies pertaining to the role of the pulmonary immune system in the pathogenesis of lung diseases. The objectives of these studies include (1) to evaluate the potential adverse effects of inhaled agents on lung immunity - specifically lung macrophages and lung interstitial lymphocytes; (2) if possible, to examine potential mechanisms of toxicity; and (3) to relate these observed changes in immune function to clinicians and regulatory agencies so that improved treatment and monitoring may be facilitated.

Studies were performed in the following areas: (1) descriptive lung immunotoxicity studies following exposure to asbestos; and (2) mechanistic studies on the role of specific cell types in the pathogenesis of asbestos related lung disease.

In studies designed to elucidate the potential immunopathogenic role of pulmonary epithelial cells in the response to asbestos, the fibrogenic fibers, chrysotile and crocidolite asbestos were shown to directly stimulate a human pulmonary type-II epithelial cell line (A549) to elicit the neutrophil chemotactic cytokine interleukin-8 in the absence of other known stimuli, suggesting an important effector role for these cells in asbestos induced disease.

Studies employing T-cell deficient mice to assess the role of cell mediated immunity in asbestos were concluded. These data suggest that T cells directly or indirectly influence the termination of a neutrophilic response and fibronectin release which ultimately influences the amount of collagen accumulation in the lung and are consistent with a protective role for T lymphocytes. Together, these studies point to a significant role for immune system elements in asbestos-related diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21149-02 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Nasal Toxicity of Glutaraldehyde

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Frank W. Kari, Ph.D. Toxicologist STB NIEHS

COOPERATING UNITS (if any)

Chemical Industry Institute of Toxicology

LAB/BRANCH

Systems Toxicity Branch

SECTION

Developmental and Reproductive Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This is a collaboration between NIEHS and the Chemical Industry Institute of Toxicology (CIIT). The objectives of this project are: 1) to generally describe and characterize the toxicity of glutaraldehyde following administration to rats and mice by inhalation; 2) to obtain quantitation of cell proliferation in the upper respiratory tract of animals exposed to glutaraldehyde; and 3) to write a comprehensive review article on the chemistry and toxicity of glutaraldehyde. This work has been completed. Results of our findings have been peer-reviewed, and reports and manuscripts have been submitted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21151-02 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Neuroimmunomodulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Virginia M. Sanders Pharmacologist STB NIEHS

Others: M. Luster Research Microbiologist STB NIEHS
J. Hong Pharmacologist LMIN NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Systems Toxicity Branch

SECTION

Immunotoxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle, North Carolina 27709

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

It has been proposed that environmental factors may alter immune function indirectly by affecting other organ systems that impact on immune function. Results have shown that norepinephrine, via beta-2-adrenoceptor stimulation, increases the number of antigen-specific B lymphocyte precursors differentiating into antibody-secreting cells, without affecting the number of antibody-secreting cells produced by each precursor. In addition, as part of a collaborative project with LMIN, results have shown that T and B lymphocyte function is altered after exposure to neuropeptide fragments derived from proenkephalin, and that both T and B lymphocytes produce and secrete the precursor peptide. Thus, a xenobiotic-induced alteration of one organ system may precipitate dysfunction in another by affecting the secretion of multi-targeted modulatory molecules. To address these questions in vivo, an animal model has been established that utilizes the scid mouse reconstituted with the same lymphocytes used in vitro. Preliminary results have shown that these mice secrete antibody after primary immunization and survive neurotransmitter depletion with 6-hydroxydopamine. These studies have implications for basic biomedical and clinical research on brain-immune interactions. An understanding of integrated system function will contribute to an increased understanding of immune and neuroendocrine function, and will contribute to the development of targeted drug strategies to prevent toxicities that may result from exposure of an integrated biological system to environmental factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21152-02 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Altered Energetics as a Mechanism of Developmental Toxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. : E. Sidney Hunter, III Toxicologist STB NIEHS

Others: Janet Allen Biological Lab Technician STB NIEHS

COOPERATING UNITS (if any)

Comparative Medicine Branch, DIR

LAB/BRANCH

Systems Toxicity Branch

SECTION

Developmental and Reproductive Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina, 27709

TOTAL STAFF YEARS:

0.65

PROFESSIONAL:

0.4

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unswelled type. Do not exceed the space provided.)

This project is designed to evaluate the potential developmental toxicity of perturbations in embryonic energy producing pathways using the whole embryo culture technique. The whole embryo culture system supports the normal growth and development of rodent embryos in an in vitro system and allows for the evaluation of the effects of an agent on the developmental processes. A number of metabolic inhibitors (2-deoxyglucose, 3-chlorolactaldehyde, dichloroacetate, fluoroacetate, iodoacetate, rhodamine 123, dinitrophenol, dicyclohexylcarbonyldiimide, and cyanide) will be used in order to produce specific metabolic perturbations in the conceptus during the critical period of organogenesis. The relationship between specific metabolic perturbations and energy production and homeostasis will be determined. Additionally, the relationships between the biochemical effects and the resulting morphological effects of these treatments will be evaluated.

Another aspect of this project will be to evaluate the mechanisms associated with biochemical maturation that occurs in the conceptus during the period of organogenesis. This project will involve an analysis of metabolic changes throughout organogenesis and the enzymatic sequelae related to the changing metabolism during development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21153-02 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Mechanisms of Developmental Toxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: E. Sidney Hunter, III, Ph.D. Toxicologist STB NIEHS

Others: Jennifer Tugman Stay in School STB NIEHS
Sonia Tabicova, Ph.D. Fogarty Fellow STB NIEHS

COOPERATING UNITS (If any)

Comparative Medicine Branch, DIR

LAB/BRANCH

Systems Toxicity Branch

SECTION

Developmental and Reproductive Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina, 27709

TOTAL STAFF YEARS:

1.6

PROFESSIONAL:

0.1

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

This project will evaluate the potential developmental toxicity of xenobiotics using the whole embryo culture system. This system supports the normal growth and development of rodent embryos in an in vitro system and allows for the evaluation of the effects of an agent on morphological, biochemical and molecular processes.

The adverse developmental effects (e.g. in utero lethality and neural tube defects) of several elemental ions, e.g. group IIIA metals (In, Ga) and arsenic have been shown in vivo. However, whether these effects are due to the direct effects of the metals or mediated through an effect on maternal homeostasis is unclear.

Preliminary studies will address the direct effects of these agents on the conceptus, and mechanistic studies will focus on free radical generation and other biochemical effects of the agents.

The mechanisms responsible for the production of cell death will be evaluated in a limb bud culture system. For these studies, the molecular and cellular effects of xenobiotics will be determined following exposure to specific agents such as retinoic acid and oxygen radical generating systems. Preliminary evidence suggests that abnormal digit formation occurs as a result of excess oxygen which is similar to the effects of many toxicants in vivo. This may suggest that the generation of oxygen radical ions may be a common mechanism of developmental toxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 ES 21154-02 STB																				
PERIOD COVERED October 1, 1991 to September 30, 1992																						
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cellular Indicators of Neuronal Insult																						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">P.I.:</td> <td style="width: 33%;">G. Jean Harry, Ph.D.</td> <td style="width: 33%;">Toxicologist</td> <td style="width: 10%;">STB</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>Others:</td> <td>Pushpa Tandon, Ph.D.</td> <td>Guest Researcher</td> <td>STB</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>Prasada Kodavanti, Ph.D.</td> <td>Guest Researcher</td> <td>STB</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>Hayes Brown</td> <td>Biologist</td> <td>STB</td> <td>NIEHS</td> </tr> </table>			P.I.:	G. Jean Harry, Ph.D.	Toxicologist	STB	NIEHS	Others:	Pushpa Tandon, Ph.D.	Guest Researcher	STB	NIEHS		Prasada Kodavanti, Ph.D.	Guest Researcher	STB	NIEHS		Hayes Brown	Biologist	STB	NIEHS
P.I.:	G. Jean Harry, Ph.D.	Toxicologist	STB	NIEHS																		
Others:	Pushpa Tandon, Ph.D.	Guest Researcher	STB	NIEHS																		
	Prasada Kodavanti, Ph.D.	Guest Researcher	STB	NIEHS																		
	Hayes Brown	Biologist	STB	NIEHS																		
COOPERATING UNITS (if any) Program Resources Group, DTRT Comparative Medicine Branch, DIR																						
LAB/BRANCH Systems Toxicity Branch																						
SECTION Developmental and Reproductive Toxicology																						
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709																						
TOTAL STAFF YEARS: 1.4	PROFESSIONAL: 1.0	OTHER: 0.4																				
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☐ (a1) Minors ☐ (a2) Interviews																						
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) Numerous receptor systems in various brain regions have been reported to be affected by toxicant exposure. This project will examine the role of toxicant-induced changes in receptors to functional changes in the cell such as the ability to maintain calcium homeostasis. This process plays a critical regulatory role in cell-cell communication and signaling. A disruption in intracellular calcium can disrupt the calcium-mediated signal transduction processes resulting in a cascade of intracellular effects. Intracellular calcium has been examined in subcellular components of the nervous system following exposure to environmental agents such as inorganic lead and polychlorinated biphenyls. Using a primary cell culture system of cerebellar granule cells, this project has examined the effects of PCBs on cellular calcium homeostasis in both a population of cells as well as individual cellular responses. Initial studies have examined the developmental ontogeny of the second messenger system in the visual system. Alterations in this profile will be studied as a measure of visual sensory functioning in the developing animal following exposure to an environmental agent. Immunohistological studies are underway to examine alterations in neuronal distribution of a calcium binding protein, Calbindin 28K, and its potential role in determining the cellular pattern of perturbation in the nervous system either following exposure to an environmental agent or during the aging process.																						

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21155-02 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Gene Expression as an Injury Target Site of Environmental Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

P.I.: G. Jean Harry, Ph.D. Toxicologist STB NIEHS

Others: Nasser Zawia, Ph.D. Staff Fellow STB NIEHS
Hayes Brown Biologist STB NIEHS

COOPERATING UNITS (if any)

Program Resources Group, DTRT
Comparative Medicine Branch, DIR

LAB/BRANCH

Systems Toxicity Branch

SECTION

Developmental and Reproductive Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

1.8

PROFESSIONAL:

1.2

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The expression of many genes is under the control of "regulatory genes" whose products are required as transcription factors to selectively turn on and off gene expression. This project will evaluate the potential effects of specific environmental agents on these transcription factors and how such effects may influence changes in target genes and subsequent gene products. The developing nervous system expresses a very high number of genes which are necessary to bring about the successful execution of the developmental program. Any alterations in this process could have a major impact on the developmental cascade of the nervous system.

This project will be conducted in vivo (in the postnatally developing rat) and in vitro (primary cell cultures of cerebellar granule cells and oligodendroglia). In situ hybridization and immunohistochemical analysis will be conducted to determine the relationship between different cell types in the nervous system and the subsequent alterations following toxicant exposure which may account for the observed neurotoxic effect. During the developmental process, genes which play a critical role as transcription factors may be regulated differently than in the mature nervous system. This project will examine the possible differences in regulation of transcription factors and the role that it may play in the differential vulnerability of the developing organism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21167-01 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Prenatal Effects of Chemicals on the Respiratory Tract

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Daniel L. Morgan	Toxicologist	STB	NIEHS
Others:	B.A. Schwetz	Supervisory Pharmacologist	STB	NIEHS
	M. P. Moorman	Engineer	STB	NIEHS
	T. Lyon	Biologist	STB	NIEHS

COOPERATING UNITS (if any)

METI

LAB/BRANCH

Systems Toxicity Branch

SECTION

Respiratory Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL STAFF YEARS:

0.55

PROFESSIONAL:

0.05

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Initial studies in this new project involve establishing a standard protocol for chemical treatment of pregnant rats with CdCl₂. These studies include determining which gestation days to treat and the appropriate dose in order to obtain an optimal effect on the fetal and neonatal respiratory tract. A prototype nose-only plethysmograph was constructed for measuring pulmonary function in neonatal rats during inhalation challenge to irritant gases. Initial testing and evaluation of this system is in progress. Methods for evaluating histological and biochemical endpoints are also being developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30106-18 STB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects of Environmental Pollutants on the Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael I. Luster	Research Microbiologist	STB NIEHS
Others:	G. Rosenthal	Toxicologist	STB NIEHS
	C. Portier	Statistician	SBB NIEHS
	S. Holladay	IRTA	STB NIEHS
	J. Wilmer	Staff Fellow	STB NIEHS
	F. Burleson	Guest Researcher	STB NIEHS
	F. Kayama	Guest Researcher	STB NIEHS
	T. Yoshida	Guest Researcher	STB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Systems Toxicity Branch

SECTION

Immunotoxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL STAFF YEARS:

6.0

PROFESSIONAL:

3.0

OTHER:

3.0

CHECK APPROPRIATE BOXES

- ☐ (a) Human subjects ☐ (b) Human tissues
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unvouched type. Do not exceed the space provided.)

Employing a variety of in vivo and in vitro techniques, the Immunotoxicology Group studies the adverse effects on the immune system resulting from occupational, inadvertent, or therapeutic exposure to drugs, environmental chemicals, and biological materials. The ongoing objectives include efforts: (1) to evaluate and examine the influence of selected drugs or environmental chemicals on the immune response and relate alterations in immunological functions with general and specific organ toxicity; (2) when applicable, to examine potential mechanisms of action at the cellular and molecular levels; (3) to relate changes in immunological functions with altered host resistance following challenge with tumor cells or infectious agents; and (4) to refine and validate a panel of immune and host resistance procedures in order to better define immunotoxic endpoints. General methodology employed includes various cell and tissue culture procedures, flow cytometry, electrophoresis (northern and western blots), hematological procedures and biochemical tests to determine the activity of immune cells. Studies have been conducted in the following areas: (a) Development and utilization of procedures to examine the effects of in utero exposure to selected environmental chemicals on the developing immune system in mice and, in particular, thymic maturation; (b) Development and utilization of model systems which allow assessment of skin immunity [i.e., keratinocytes]. Endpoints for these assessments include production of soluble mediators (cytokines), surface markers and effector cell function; (c) Implementation of a model system to examine the participation of cytokines in chemical-induced hepatotoxicity; (d) Examination of the mechanisms of chemical-induced thymic atrophy with particular emphasis on apoptotic mechanisms (programmed cell death); (e) Examining the control of cytochrome P450c and the TCDD-inducible or tumor associated aldehyde dehydrogenase in selected rat immune cell and lymphoid organs; (f) Efforts are being taken in which immunodeficient mice are being "restored" with immune cells from humans; and (g) Statistical evaluation of the immunotoxicology database.



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